EFFECT OF YERBA MATE TEA CAFFEINE AND GREEN COFFEE BY-PRODUCTS ON *IN VITRO* ADIPOGENESIS AND LIPID ACCUMULATION AND THEIR INCORPORATION INTO FUNCTIONAL BEVERAGES

BY

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THESIS

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ABSTRACT

In addition to its stimulatory effect, caffeine has been also associated with suppressing lipid accumulation and weight gain, making the well established energy drink market an ideal avenue to expand product health functionality. The objective of this study was to evaluate the effect of caffeine extracted from Yerba mate (*Ilex paraguariensis*) (matein, MT) and green coffee (*Coffea arabica*) byproducts (fractions GC1, GC2, GC3 and GC4) on inhibition of lipid accumulation in 3T3-L1 adipocytes compared to synthetic caffeine (SC). The antioxidant capacity (AC) and total polyphenol content (TPC) of these novel caffeine sources were also investigated and compared to SC using the Oxygen Radical Absorbance (ORAC) assay and the Folin-Ciocalteu method, respectively. Lipid quantification was done by the Oil Red O staining and real time polymerase chain reaction (RT-PCR) was performed to determine expression of two genes involved in lipid metabolism, lipoprotein lipase (LPL) and fatty acid synthase (FAS). Inhibition of lipid accumulation (%) was highly correlated to caffeine concentration of the samples ($R^2 = 0.88$). MT and GC1 had the highest caffeine concentrations (90.8 and 93.8%, respectively), and exhibited the highest lipid reduction (18.3 and 17.1%, respectively) compared to untreated cells ($p<0.05$). MT and GC1 had similar inhibition of lipid accumulation compared to SC (16.3%), epigallocatechin gallate (EGCG) (17.5%), and Orlistat (17.9%). Adipocytes treated throughout differentiation resulted in an elevated inhibitory effect, with MT (22.8 ± 2.2%) and GC1 (26.8 ± 1.8%) having similar inhibitory capacity to SC (29.3 ± 0.5%). RT-PCR showed MT and GC1 may play a role in lipid metabolism by suppressing LPL and FAS. The GC byproducts and MT at 1000 µM had an AC range of 19.4 ± 0.2 to 1550.2 ± 19.2 with a mean of 446.5 Trolox µM equivalents (eq.), whereas SC had none. The TPC of the GC

byproducts, MT, and SC ranged from 5.7 ± 0.3 to 153.5 ± 1.1 gallic acid (GA) µg eq., with SC resulting in the lowest. In conclusion, these results suggest that the incorporation of MT and GC1 into beverage formulations would provide a natural source of caffeine and potentially aid in long-term weight maintenance, and give in contrast to SC, antioxidant benefits.

To my family and friends

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I. INTRODUCTION

Obesity is characterized by a chronic imbalance between energy intake and energy expenditure resulting in an enlargement in adipose tissue due to an increase in both adipocyte cell number (hyperplasia) and size (hypertrophy) (Schmid and others, 2005; Avram and others, 2007; Bays and others, 2008). Its prevalence has steadily increased worldwide and is one of the leading causes of preventable deaths due to its association with the development of a number of diseases including type II diabetes, coronary heart disease, hypertension and a heightened incidence of certain forms of cancer (Kopelman, 2000; Zimmet and Alberti, 2008). The amount of adipose tissue can be regulated through the inhibition of adipogenesis and fat accumulation (Rayalam and others, 2008). Fat accumulation in adipose tissue can be reduced by reducing lipid uptake by adipocytes through the suppression of lipoprotein lipase (LPL) or reducing lipid synthesis through inhibiting fatty acid synthase (FAS) among other mechanisms (Jing-Jing and others, 2008; Wang and Eckel, 2009). Increased research in the area of bioactive compounds and their potential role for the improvement of long term weight maintenance are needed. Weight loss is increasingly recognized to have major health benefits for overweight individuals as well as increase the life expectancy of those suffering from obesity-related health complications (Goldstein, 1992; Westerterp-Plantenga and others, 2005; Rayman and others, 2008). It has been found that modest weight loss of just 5% to 10% of initial body weight can lead to beneficial health effects (Goldstein, 1992; Wing and others, 1992; van Gaal and others, 1997).

Yerba mate (*Ilex paraguariensis*) tea is rich in caffeine and polyphenols, and has been associated with weight loss as well as also having a hypolipidemic effect

(Anderson and Fogh, 2001; Martins and others, 2009). Similarly, studies have shown that coffee bean extracts can aid in weight management and have potent anti-obesity and hypotriglyceridemic properties due, in part, to its caffeine concentration (Tanaka and others, 2009). Throughout coffee manufacturing, byproducts containing caffeine are produced which can be used as natural alternative sources to synthetic caffeine. Therefore, investigating the anti-obesity properties of natural caffeine sources would be beneficial to the food industry as well as the consumer.

Caffeine has been shown to have anti-obesity effects through the suppression of body weight gain and adipose tissue formation (Diepvens and others, 2007; Huang and others, 2009). Caffeine has also been reported to suppress body weight gain by stimulating thermogenesis, extending sympathetic stimulation, suppressing food intake and reducing adipose tissue mass (Dulloo and others, 1999, 2000; Hasegawa and Mori, 2000; Zheng and others, 2004; Kazuo and others, 2005; Kobayashi-Hattori and others, 2005; Lopez-Garcia and others, 2006; Tanka and others, 2009). Thus, caffeine consumption could be an effective tool to increase the success rate of long-term weight maintenance and possible weight loss in humans.

The aim of this study was to evaluate *in vitro* the effect of caffeine, extracted from Yerba mate tea (matein, MT) and green coffee byproducts (fractions GC1, GC2, GC3 and GC4), on lipid accumulation, and FAS and LPL gene expression using 3T3-L1 adipocytes, in comparison to synthetic caffeine (SC). Furthermore, the antioxidant capacity (AC) and the total polyphenol content (TPC) of these natural sources of caffeine were analyzed and compared to SC. The final objective was to replace SC in beverage applications with MT and GC byproducts.

II. LITERATURE REVIEW

1. Energy Drinks

1.1 Market Size

Energy drinks fall into the category of functional beverages, which also encompasses sports drinks and nutraceutical beverages (Datamonitor, 2008). Energy drinks refer to beverages that contain, besides calories, caffeine in combination with purportedly energy-enhancing ingredients such as taurine, herbal extracts, and B vitamins, with the advertised purpose of providing the consumer with increased energy. Energy drinks do not emphasize energy derived from the calories they contain, but rather through their caffeine or herbal supplement content. Sports drinks are designed to be consumed before or during exercise to prevent dehydration, supply carbohydrates, and provide electrolytes and they typically do not contain caffeine (Coombes and Hamilton, 2000). Nutraceutical beverages on the other hand are designed to promote and enhance health, usually by containing bioactive compounds such as concentrated extracts from teas, fruits and vegetable or herbs. Additionally, some nutraceutical beverages are fortified with vitamins and minerals and contain significant levels of antioxidants and polyphenols. In some instances energy drinks could overlay into the nutraceutical beverage category depending on their ingredient composition. The energy drink segment encompasses an array of options including ready-to-drink, shots and powder forms of the beverages.

Energy drinks first appeared in Europe and Asia in the 1960s in response to public demand for a dietary supplement that would result in increased energy (Reissig and others, 2009). In 1962 a Japanese company, Taisho Pharmaceuticals, met that

demand by launching Lipovitan D, one of the very first energy drinks, which still dominates the Japanese market. Lipovitan D contains B vitamins, taurine, and ginseng, which are all frequent constituents of mainstream energy drinks, with the intended purpose of providing the consumer with sustained energy to reduce mental and physical fatigue (Taisho Pharmaceutical Co. Ltd., 2009). Energy drinks did not make their way into the U.S. market until 1997 when Red Bull was first introduced, which originated and was initially launched 10 years earlier in Austria (Reissig and others, 2009). Since the 1960's, the energy drink market has grown into a multibillion dollar industry. Globally, energy drinks hold 47.3% of the functional beverage's overall market share, while in the U.S. energy drinks comprise 62.6% of this market share (Datamonitor, 2008). Energy drinks in particular have seen impressive growth in the U.S., as well as abroad. In 2008, the functional beverage industry reached global sales of $26.9 billion with a compound annual growth rate (CAGR) of 8.6% from 2004-2008. The U.S. contributed significantly to the functional beverage industry's total, accounting for $7.6 billion in revenue and a CAGR of 20.6%. In addition, the U.S. energy drink industry is forecasted to more than double and reach an astounding $19.7 billion in 2013, which is almost a 160% increase from 2008 (Datamonitor, 2008). Within the functional beverage category, the energy drink segment has seen the largest volume growth and increased annual sales both in the U.S. and abroad, taking in $4.8 billion in 2008 in the U.S. alone (Datamonitor, 2008). This increased growth can be attributed to more private label initiatives, larger container sizes, multi-pack options, sugar free versions, and juice hybrids that have a more palatable flavor (GMID, 2008; Canadean, 2009). Currently, there are more than 300 varieties of energy drinks from more than 200 brands in the U.S. alone all purporting to

increase energy, longevity and vitality in some form or another (Energyfiend, 2009). Although there is an abundance of energy drinks to choose from, the majority of the market share is comprised by only a handful of varieties, with Red Bull, Monster, Rockstar, Full Throttle, and Amp, with Red Bull accounting for 42% of the market share (Beverage Spectrum, 2008). All in all, the energy drink industry has proven extremely profitable and is anticipated to continue with this same success in the years to come, and will be seen with new and innovative product launches reaching a more expanded market.

1.2 Target Demographic

Athletes were initially the primary consumers of energy drinks. However, as the energy drink market has grown and expanded into various niche markets, athletes are no longer the primary target. Today, the majority of energy drinks are targeted at teens and young adults 18-34 years old due to this generation's on-the-go lifestyle and receptiveness to advertisements for these types of products (Lal, 2007). The popularity of energy drinks among the younger generation is evidenced by 34% of 18 to 24-year-olds being regular energy drink users (Mintel, 2009; O'Brien and others, 2008). Another report found that about one-half of college students consume at least one energy drink per month in the hope to increase their energy level, to compensate for a lack of sleep or to mix with alcohol (Miller 2008). The marketing and branding of many energy drinks reflects the market to which these companies are targeting. A review presented at the 2007 IFE Americas Food and Beverage Show confirmed that energy drink companies' primary target market was adolescents and young adults (Agriculture and Agri-Food Canada, 2008). Their appraisal stated that many of the energy drink companies were using cross-promotional tactics to reach their consumer base by integrating their product with extreme sporting events, such as the X-games or NASCAR, as well as advertising

their product in connection with popular music icons. In addition to those tactics, energy drink companies have begun using creative, and in some cases defiant, names for their products to draw in consumers, with some examples being Full Throttle, Ammo, Havoc, Hydrive, Monster, and Morning Spark (Energyfiend, 2009). The target market for energy drinks is broadening as new products are developed in an effort to reach niche sub-markets and differentiate themselves from their competition. Such sub-markets include energy drinks just for women, the carbohydrate-conscious, bodybuilders, or extreme sports enthusiasts. In 2007, Del Monte Foods launched its first energy drink called Bloom Energy claiming that it was formulated specifically with women in mind. Other energy drinks are targeted towards athletes such as Lucozade Sport and Revenge Sport which play up their advertised ability to increase physical performance and reduce fatigue in high-endurance sports. The developers of Energy Fizz reached consumers through a different marketing approach, which was to promote their product's convenience factor of being a powder that is packaged in a small portable tube that can be easily added to water on the go in order to get that needed energy boost. Although on-the-go drink mixes are not new to the beverage industry as a whole, within the energy drink category they are. Other energy drinks promote the unique qualities that make them stand out from the rest, such as being all natural, organic, or gluten free, as well as diabetic- or vegetarian-friendly. Energy drinks are still a developing industry in which a diverse range of new and innovative products will be seen in the years to come, with new innovations geared more towards the increasing number of health-conscious individuals.

1.3 Behavioral Impact of Energy Drink Consumption

There have been conflicting results concerning the effect of energy drinks on physiological and cognitive performance. Some studies have reported no significant difference in either the physiological or cognitive performance of individuals who have consumed energy drinks compared to those who have not (Carvajal-Sancho and Moncada-Jimenez, 2005; Umana-Alvarado and Moncada-Jimenez, 2004). Research has been aimed at determining the behavioral effects of energy drinks on consumers; specifically, on their mood, concentration, reaction time, alertness, endurance, physical performance, and risk taking. The cognitive and physiological effects after the consumption of an energy drink in comparison to a placebo resulted in significantly improved performance on both secondary memory and speed of attention (Scholey and Kennedy, 2004). Another study looked at the effect Red Bull had on cognitive performance and well-being of the studied subjects, concluding that its consumption had a positive impact (Seidl and others, 2000; Alford and others, 2001). Comparing the consumption of energy drinks to a placebo also revealed that energy drinks had an energizing effect, with the strongest effect observed 30 to 60 min after consumption, and the individuals having sustained energy for up to 90 min (Smit and others, 2004). The consumption of Red Bull also proved to be beneficial in improving aerobic endurance and anaerobic performance (Alford and others, 2001). Another study showed that consuming an energy drink 40 min before exercise can improve endurance and physical performance (Ivy and others, 2009). Improvements in visual information processing, attention, and verbal reasoning have also resulted from the consumption of energy drinks (Warburton and others, 2001). These responses are attainable from the consumption of

just one energy drink because research has shown that as little as 12.5 to 100 mg of caffeine can improve cognitive performance and mood (Smit and Rogers, 2000). Furthermore, a study looked at the effect an energy blend containing 80 mg caffeine, taurine, glucuronolactone, vitamins, and sugar on counteracting driver sleepiness and concluded that this blend was beneficial in reducing sleepiness and sleep-related driving incidents (Reyner and Horne, 2002). There have also been several studies which have looked at the association between energy drink consumption and problem behavior. The results of a recent study concluded that increased energy drink consumption was associated with increased risk-taking behaviors (Miller, 2008).

Another common practice, especially among college students, is to mix alcohol with energy drinks (O'Brien and others, 2008). It has been found that almost 25% of all college students who currently drink, mix alcohol with energy drinks (O'Brien and others, 2008). The concept of alcoholic energy drinks has been a controversial issue in which the alcohol industry has received much criticism due to the dangers associated with this trend. Several studies have shown that the consumption of energy drinks in combination with alcohol has resulted in a decreased level of perceived intoxication, which could result in an increased number of driving accidents or other alcohol-related incidents (Ferreira and others, 2006; Marczinski and Fillmore, 2006). The masking effect that energy drinks may have over alcohol intoxication is due to the combination of the stimulatory effect of caffeine and the depressant effect of alcohol on the body, hindering an individual's true awareness of the typical signs of alcohol intoxication (eg. drowsiness) (Oteri and others, 2007). However, one study found no significant differences among subjects who consumed alcohol in combination with energy drinks

and those who just consumed alcohol (Ferreira and others, 2004). Further research is needed to better understand this association as well as increased awareness of the potential dangers that could result from the combination of alcohol and caffeinated beverages.

1.4 Active Ingredients in Energy Drinks

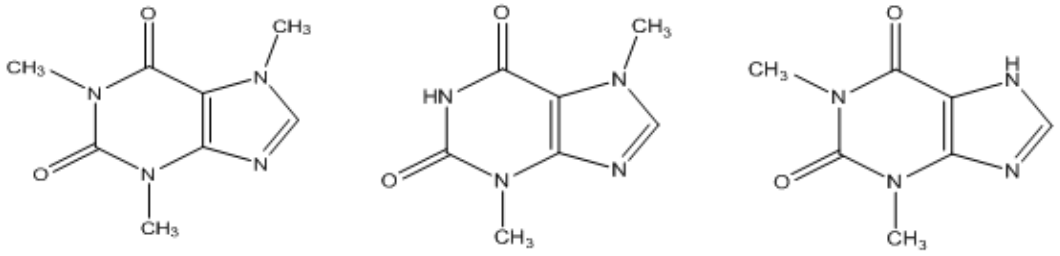
Although there are hundreds of energy drinks out in the market, many share very similar ingredient profiles with the primary ingredient being caffeine. Many of these unique energy blends consist of caffeine, taurine, and B vitamins, in various combinations, as well as at varying concentrations to act as the stimulants in those beverages. **Figure 1** depicts the chemical structures of the more widely used active ingredients found in mainstream energy drinks. In addition to those stimulants, sugar is typically added, although many brands have sugar free options available as well. Other popular ingredients that are added to energy drinks are ginseng, guarana and yerba mate. The various ingredient combinations are critical because they are what give the beverage its distinct overall flavor, the amount of energy that will be produced, as well as the duration of that energy, and the various health properties of the beverage.

Caffeine

The majority of energy drinks contain caffeine as an active ingredient due to its stimulatory effect on the central nervous system. Caffeine's main mechanism of action in concentrations typically achieved after the consumption of a caffeinated beverage is to act as an adenosine receptor blocker in the brain (Dunwiddie and Mansino, 2001; Pettenuzzo and others, 2008). Caffeine has a similar chemical structure to that of adenosine, allowing caffeine to mimic adenosine and attach to the adenosine receptors. The blockage of adenosine to the neurons causes the sleep promoting effects of adenosine

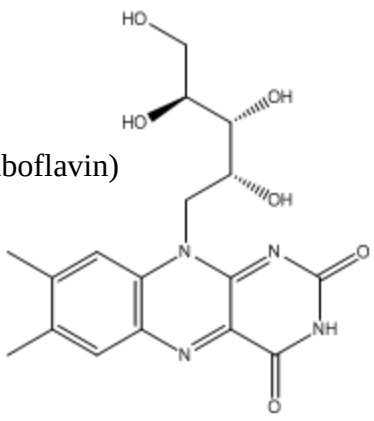
A

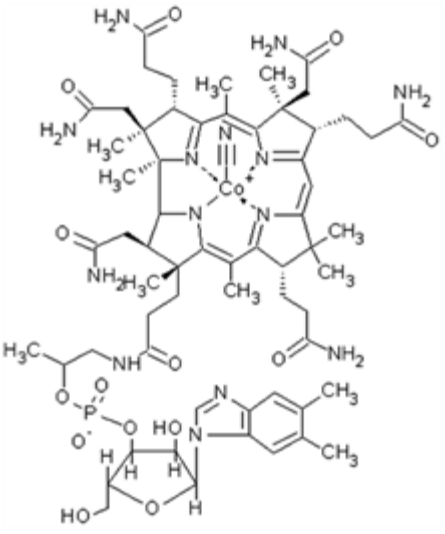
a. b. c.



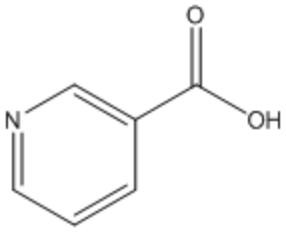
B

Vitamin B-2 (riboflavin)

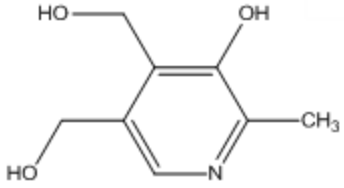




Vitamin B-12



Vitamin B-3 (niacin)



Vitamin B-6

C

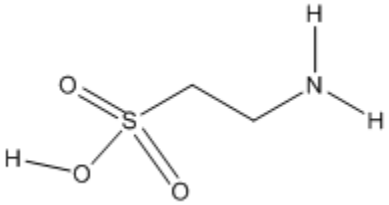


Figure 1. Chemical structures of active ingredients commonly found in energy drinks. (**A**) Major components of guarana: (a) caffeine, (b) theobromine and (c) theophylline; (**B**) B-vitamins (Helmenstine, 2009); (**C**) Taurine.

to stop, resulting in the neurons speeding up instead of slowing down (Ferre, 2008). Caffeine is also known to increase the secretion of epinephrine, which can lead to a variety of secondary metabolic changes that can positively affect physical or mental performance (Graham, 2001). Caffeine has been widely studied in a variety of areas regarding human health and performance, and it is evident that caffeine consumption can increase energy utilization (Smit and Rogers, 2002). Several studies also confirm caffeine's ability to enhance mood and alertness (Kaplan and others, 1997; Smit and Rogers, 2002; Lorist and Tops, 2003), exercise performance (Doherty and Smith, 2004; Graham, 2001), the speed at which information is processed, awareness, attention, and reaction time (Cysneiros and others, 2007). Caffeine also has a stimulatory effect on thermogenesis (Acheson and others, 1980; Dulloo and others, 1989; Astrup and others, 1990; Bracco and others, 1995). In addition, caffeine consumption has also been linked to reduced food intake (Tremblay and others, 1988; Racotta and others, 1994; Lima and others, 2005) and to promote lipolysis in both animals and humans (Hasegawa and Mori, 2000; Zheng and others, 2004; Kobayashi-Hattori and others, 2005; Lopez-Garcia and others, 2006).

A research review regarding caffeine consumption concluded that among the healthy adult population, a moderate daily caffeine intake of $\leq$ 400 mg (equivalent to 6.5 mg/kg bw/d for a 70-kg person) was not associated with any adverse effects (Nawrot and others, 2003). These recommendations were based primarily on published human data obtained through a comprehensive literature search made on the basis of no adverse effects such as general toxicity, cardiovascular effects, effects on bone status and calcium balance (with consumption of adequate calcium), changes in adult behavior, increased

incidence of cancer and effects on male fertility (Nawrot and others 2003). This review also concluded that pregnant woman should limit their caffeine intake to ≤ 300 mg/d (equivalent to 5 mg/kg bw/d for a 70 kg person) and children should consume ≤ 2.5 mg/kg bw/d (Nawrot and tohers 2003). In children, controlled clinical trials showed no adverse effects when consuming caffeine at levels up to 3 mg/kg bw/d (Higdon and Frei 2006). The exact amount of caffeine necessary to produce any adverse effects, such as sleeplessness, irritability, increased urination, abnormal heart rhythms or stomach upset, varies from person to person depending on their weight and sensitivity to caffeine (Higdon and Frei 2006). **Figure 2** shows a comparison of the amount of caffeine found in commonly consumed beverages, where it is apparent that the caffeine concentration of

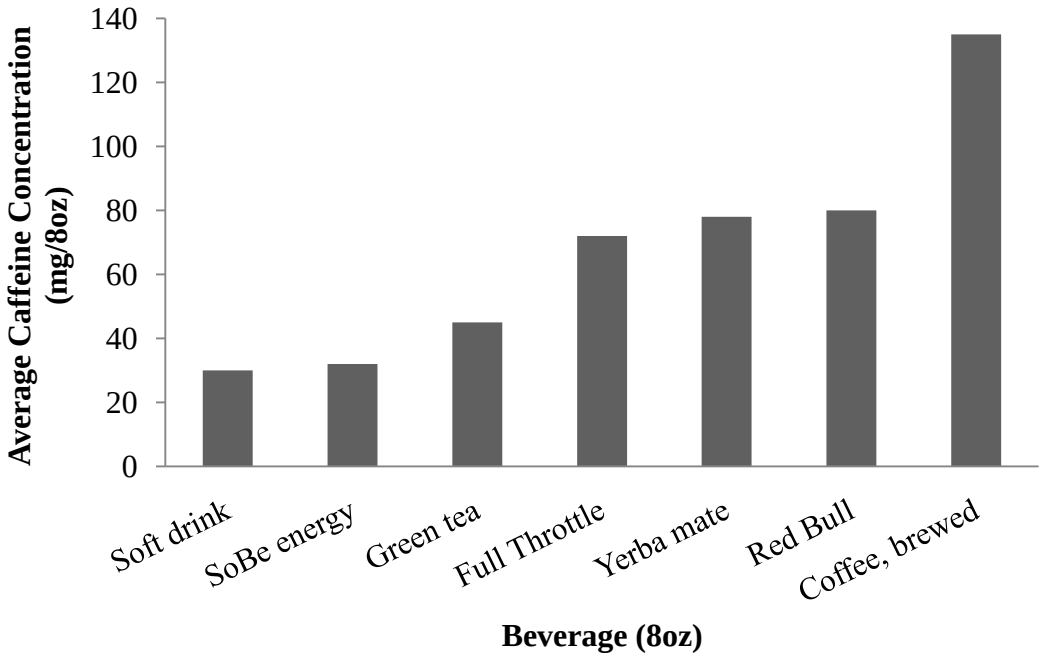


Figure 2. Caffeine (mg/8 oz) comparison in a variety of beverages

the average mainstream energy drink contains significantly less caffeine than the ≤ 400 mg/d reported as the safe level (Nawrot and others, 2003). The average caffeine concentration of an energy drink typically ranges from 80-140 mg/8 oz, well below this ≤ 400 mg per day limit and is comparable to consuming 5 ounces of coffee or 2 cans of a caffeinated soft drink (Malinauskas and others, 2007). However, it is important to be aware of the serving size since many of the 16-oz containers hold 2 servings. Caffeine also has a diuretic effect regardless if its consumption is through an energy drink, tea, or coffee (Riesenhuber and others, 2006). There are, however, several studies which conclude that caffeine consumption at physiologically relevant dosages (≤ 400 mg/d) does not cause diuresis (Falk and others, 1990; Scott and others, 2004; Armstrong and others, 2007). Caffeine has a long history of safe use and overwhelming scientific evidence maintains that when consumed in moderation (300-600 mg/day/adult) no adverse effects should occur. Nonetheless, caution should be taken in regards to the amount of caffeine consumed per day.

Taurine

Taurine (2-aminoethyl sulfonic acid) is a sulfur containing amino acid that is the most abundant amino acid found naturally in our bodies, primarily in the retina and skeletal and cardiac muscle tissue (Timbrell and others, 1995; Imagawa and others, 2009). Taurine is derived from the metabolism of methionine and cysteine (Huxtable, 1992; Stipanuk, 2004). It is also present in common food items such as meat and fish. It has been estimated that the average daily human intake of taurine is between 40-400 mg, making taurine supplementation unnecessary unless a strict vegetarian diet is being followed with no meat or fish consumption (Shao and Hathcock, 2008). The

incorporation of taurine into energy drinks and other products has increased a great deal over the past 10 years with taurine also being one of the most extensively used and studied amino acids (Shao and Hathcock, 2008). However, when energy drinks launched between 2004 and 2008 were evaluated for the presence of taurine, a reduction in taurine was seen. The results showed that one in four (27%) energy drinks in 2004 contained taurine, whereas in 2008 it was reduced to one in five (21%) (Mintel, 2009).

Taurine is associated with a variety of physiological functions including neuromodulation, cellular membrane stability, and modulation of intracellular calcium levels which has been seen both *in vitro* and *in vivo* (Huxtable, 1992; Timbrell and others, 1995). Further research needs to be done on taurine to better explain the underlying mechanisms of action. Furthermore, taurine has been seen to enhance endurance performance and to aid in the reduction of lactic acid buildup after exercise (Matsuzaki and others, 2002; Imagawa and others, 2009).

Once ingested, taurine experiences minimal metabolism in the body with taurine mainly undergoing conjugation to form bile salts as well as degradation to sulfate (Munro and Renwick, 2006). The minimal metabolism of taurine allows for larger dietary intakes without any interference with the metabolism of other compounds in the body, and excess taurine is eliminated unchanged in the urine (Munro and Renwick, 2006).

The synthetic taurine that is present in energy drinks is found in very large concentrations. Taurine analysis of 80 different energy drinks showed an average concentration of 3180 mg/L which is equivalent to 753 mg/8 oz (Triebel and others, 2007). Several studies have determined the effect of taurine at various dosages ranging

from 375 mg/d to 8000 mg/d, concluding that there are no adverse effects (Mantovani and de Vivo, 1979; Kendler, 1989; Ikeda, 1997). Other studies have also looked into the safety of taurine supplementation in humans and found no adverse effects (Sirdah and others, 2002; Brons and others, 2004; Zhang and others, 2004). Although there has been no evidence showing taurine to cause any adverse health effects, concern has been raised since little research has been conducted on the effects of large quantities of taurine in combination with other ingredients commonly found in energy drinks such as caffeine.

Guarana

Guarana comes from the *Paullinia cupana* plant, indigenous to South America. It originated in the Amazon basin in Brazil, where it has had a long history of use (Angelo and others, 2008). It is commonly known for its small-berry like fruit it produces, which contains one to three dark seeds, accounting for the only edible part of the guarana plant (Scholey and Haskell, 2008). The seeds contain a significant amount of caffeine, with 1 g guarana seed being equivalent to about 40 mg caffeine (Finnegan, 2003). Guarana contains other xanthine alkaloids specifically theobromine and theophylline, however, at much lower levels compared to caffeine (Weckerle and others, 2003). In addition to the caffeine content, guarana also contains relatively high amounts of saponins, flavonoids, and tannins, all contributing to the plants bioactive properties including its antioxidant activity (Espinola and others, 1997; Mattei and others, 1998). Guarana has become an increasingly common natural additive in energy drinks in recent years, largely for its stimulatory effect (Scholey and Haskell, 2008). It has been stated that the caffeine from guarana is released at a slower rate compared to pure caffeine, giving off a more subtle and lengthier stimulatory effect (Scholey and Haskell, 2008). It

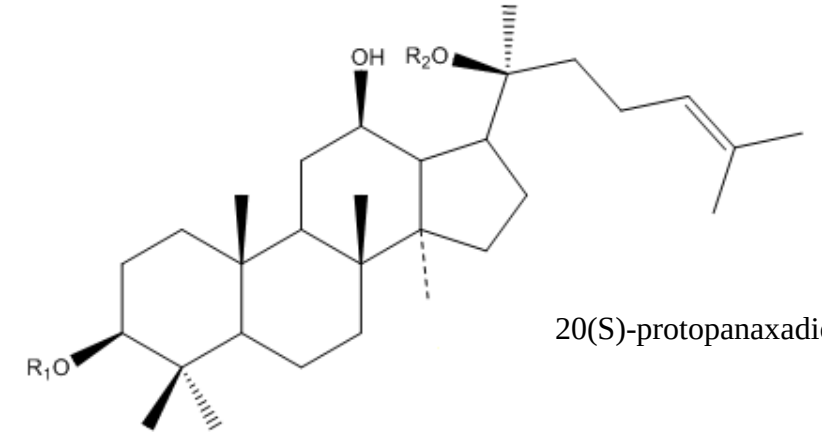
is purported that this slower release is due to guarana being insoluble in water as well as containing tannins and saponins (Edwards and others, 2005). There is however, no conclusive research that shows the caffeine release and absorption from guarana to be any different from that of pure caffeine (Bempong and Houghton, 1992). Guarana has been suggested to improve cognitive performance, mental fatigue, and mood all at physiologically relevant dosages (≤ 400 mg/d), an effect supported by several research studies (Haskell and others, 2007; Kennedy and Haskell, 2008; Scholey and Haskell, 2008). Guarana has also been associated with inducing lipid metabolism, found to be due to its methylxanthine content (Lima and others, 2005). Additionally, guarana has been shown to exert no toxic effects when consumed both in acute high dosages as well as in chronic lower dosages (Mattei and others, 1998).

Ginseng

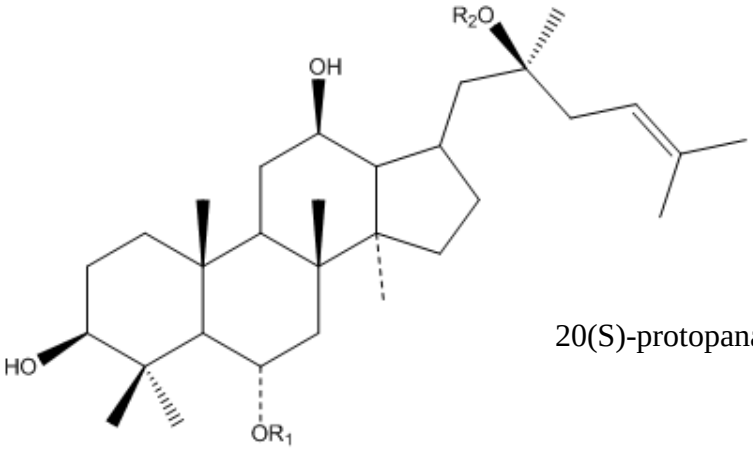
The ginseng root has been used for over 2,000 years by many people in East Asian countries including China, Japan and Korea as a medicine for various diseases and for promoting longevity (Lee and others, 2005; Nam and others, 2005). The ginseng plant is a small, shade-loving perennial shrub that reaches about 60 cm in height and belongs to the plant family Araliacae. The market for ginseng products and ginseng-related products has been estimated at $3.5 billion worldwide and is forecast to increase as a result of the increased research on the herb's pharmacological effects (Hong and others, 2006). There are several reported health benefits of ginseng which includes being an immune stimulant, producing improved physical and mental conditions, and having antistress, antiaging, antioxidant, and anti-inflammatory properties (Coon and Earnst, 2002; Lu and others, 2009). The pharmacological properties of ginseng are attributed to

its active constituents with ginsenosides being the most biologically active accounting for 2-3% of ginseng (Okazaki and others, 2006; Lu and others, 2009). **Figure 3** shows the chemical structures of selected ginsenosides that were isolated from *Panax ginseng* (Kim and others, 2007). Ginsenosides are secondary metabolites from ginseng root. They are triterpene saponins and more than 40 of these compounds have been isolated and identified (Nah and others, 2007). Each ginsenoside has its own unique structure, therefore, various pharmacological effects can result (Lu and others, 2009). Many studies have investigated a single purified ginsenoside rather than analyzing a ginseng root extract in its entirety to determine and explain the various mechanisms of action of ginseng (Zhou and others, 2004; Cheng and others, 2005; Hofseth and Wargovich, 2007). Ginsenosides have been found to inhibit reactive oxygen species production, stimulate nitric oxide production, improve central nervous system function, and prevent cardiovascular and other diseases (Lu and others, 2009).

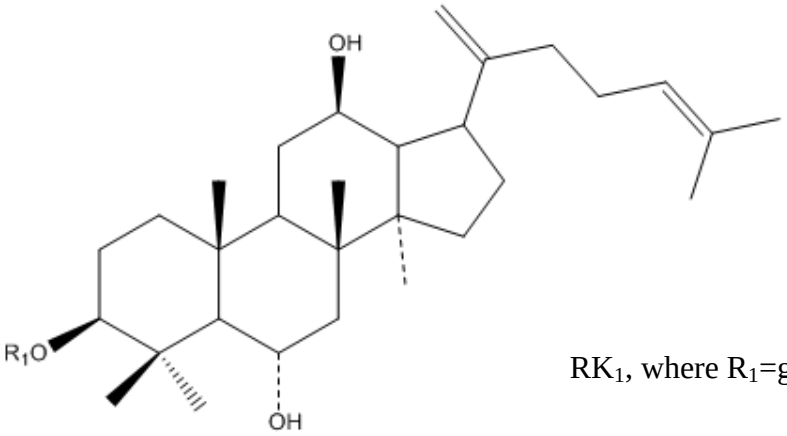
In view of the increasing popularity of ginseng and its reported pharmacological effects, it is important to know whether or not there are any health risks for the consuming public. Based on several studies conducted with animals and humans, ginseng is generally considered safe (Coon and Earnst, 2002). However, the use of ginseng in very high doses has resulted in some side effects, which included hypertension, diarrhea, and sleep disturbances (Coon and Earnst, 2002). Nevertheless, many other studies have claimed that, in comparison with other phytomedicines, ginseng has not been shown to produce serious side effects or dangerous interactions with other drugs (Nah and others, 2007).



20(S)-protopanaxadiol, where R_1=R_2=H



20(S)-protopanaxadiol, where R_1=R_2=H



RK_1, where R_1=glucose[2]-glucose

Figure 3. Chemical structures of selected ginsenosides isolated from *Panax ginseng*, an active ingredient commonly found in energy drinks. (Adapted from Kim and others, 2007)

Yerba Mate

Yerba mate (*Ilex paraguariensis*) is native to South America where its main function is the production of yerba mate tea (Heck and de Mejia, 2007). Yerba mate tea is a commonly consumed beverage in some South American countries and has been for centuries; however, it is increasing in popularity globally due to its content of a variety of bioactive components including polyphenols, xanthines, flavonoids, saponins, amino acids, minerals, and vitamins (Heck and de Mejia, 2007). The abundant array of phytochemicals present in yerba mate has been connected to various health benefits. Yerba mate possesses anticancer, anti-inflammatory, and antidiabetic properties as well as acts as an inhibitor to oxidative stress (Heck and de Mejia, 2007; Markowicz-Bastos and others, 2007). Moreover, yerba mate has shown to have high cytotoxicity to cancer cells and strong inhibition against topoisomerase II, which plays a role in cell division and therefore works to inhibit cancer cell proliferation (Heck and de Mejia, 2007). Yerba mate also has a positive impact on the management of obesity, both *in vivo* and *in vitro* (Pang and Choi, 2008; Arcari and others, 2009; Martins and others, 2009). The consumption of yerba mate significantly improved the serum lipid parameters in normolipidemic and dyslipidemic individuals (de Morais and others, 2009). Furthermore, yerba mate increased the degree of reduction in LDL-cholesterol levels in individuals who were also under statin therapy (de Morais and others, 2009).

In addition, yerba mate is a central nervous system stimulant due to its high caffeine concentration, which is the primary reason for yerba mate to be incorporated into energy drink formulations. The caffeine concentration in 1 cup (8 oz) of yerba mate tea is equivalent to about 78 mg, which is approximately equal to the amount in an 8 oz Red Bull (80 mg) (Heck and de Mejia, 2007).

On the other hand, concerns have been raised regarding an association between yerba mate and the occurrence of certain types of cancer, specifically oral, esophageal, lung, bladder, and renal (Heck and de Mejia, 2007). However, there is no conclusive evidence that this association is a result of the consumption of yerba mate, rather than due to various lifestyle choices including smoking and excessive alcohol consumption (Heck and de Mejia, 2007). In addition, these cases have primarily been reported in areas of South America where large amounts of yerba mate are consumed at very hot and damaging temperatures which could lead to increased absorption of carcinogens that are found in cigarette smoke or other environmental pollutants (Heck and de Mejia, 2007).

B Vitamins

B vitamins are a group of eight individual water-soluble vitamins, usually referred to as the B complex when grouped together, and all play essential roles in cellular processes. B vitamins are incorporated into many of the mainstream energy drinks such as Red Bull where a can contains 360% of the recommended daily allowance (RDA) of B-6, 120% of B-12, and 120% of B-3. The addition of excess amounts of B vitamins is also seen in the more extreme energy drinks like 5-Hour Energy shot which contains 8,333% of the RDA for vitamin B-12 and 2,000% of the RDA for B-6. It is claimed that the consumption of these large amounts of B vitamins increases mental alertness and focus, as well as improve mood. The average person, however, consumes the RDA of B vitamins from a typical diet since B vitamins are found in a variety of foods including bananas, lentils, potatoes, tuna, and turkey. Vitamins B-2 (riboflavin), B-3 (niacin), B-6 (pyridoxine, pyridoxal, pyridoxamine), and B-12 are the most common of the B vitamins that are incorporated into energy drink formulations. Vitamin B-2 is a

coenzyme in the metabolism of carbohydrates. Vitamin B-3 plays a major role as a coenzyme in energy metabolism, fat synthesis, and fat breakdown (Wardlaw and Smith, 2009). Vitamin B-6 is a group of 3 structurally similar compounds that all can be converted into the vitamin B-6 coenzyme which aids in the utilization of carbohydrates, fats, and proteins. Vitamin B-12 assists in folate metabolism and in nerve function (Wardlaw and Smith, 2009). Since all of the B vitamins are water-soluble, once the RDA has been met, the excess vitamins are excreted from the body via urine. Although the consumption of a large amount of B vitamins does not possess any adverse health effects, the logic behind the extreme amounts of B vitamins in these beverages is not well rationalized.

1.5 Beverage Health Functionality

In addition to the energy enhancing properties of the beverage products, many contain a variety of health promoting constituents including mainly antioxidants such as polyphenols. Products high in antioxidants are important because they help protect the body's cells from the damaging effects of free radicals which are known to damage proteins, lipids and DNA, reducing the risk of diseases such as cancer and coronary heart disease (Awika, 2003; Miller, 2006). According to market research done by Mintel, about 60% of the U.S. respondents stated that they look for antioxidants when shopping for functional beverages, which definitely is an area in which energy drink companies could capitalize on (Lal, 2007). Increasing the antioxidant and polyphenol content of energy drinks could be a key driver to increased sales and can be accomplished by incorporating yerba mate, green tea, ginseng or fruits such as pomegranate or the maqui and acaí berry into the beverage formulation. A future trend that could prove to be very

effective would be for energy drink companies to increase the functionality of their beverages to target the growing number of health conscious consumers (Lal, 2007). Areas in which these companies could expand could be vitamin and mineral fortification, organic options, all natural, no artificial flavors, preservatives or colors, weight control formulations, incorporation of fruit, as well as continuing with the low carbohydrate and low sugar options that are already highly visible in the market.

1.6 Safety and Regulations

The regulation of energy drinks varies throughout the world with the U.S. having one of the more lax systems. The U.S. Food and Drug Administration (FDA) code of federal regulations #21CFR-182.1180, lists caffeine, a main component in energy drinks, to have generally recognized as safe (GRAS) status which is a result of an overwhelming amount of scientific evidence concluding that when consumed in moderation ($\leq$ 400 mg/d) caffeine has no adverse health effects. The acute toxic level for the consumption of caffeine is not well established, but for adults it is approximately 10 g/d, which is comparable to consuming approximately 100 cups of coffee per day, an excessive amount (Nawrot, 2003). The FDA does regulate the caffeine content of cola drinks, allowing 0.02% of caffeine, totaling 71 mg for a 12 oz soft drink, to be added to those beverages; however that parameter does not cross over to energy drinks (U.S. code of federal regulations, #21CFR-182.1180). In the U.S., energy drink companies have free reign over the caffeine content of their beverages because the FDA has placed no restrictions on an upper limit for caffeine. Scientific as well as public concern has developed due to the increasing numbers of extreme energy drinks entering the market having caffeine concentrations well above those of mainstream energy drinks, which

contain on average 10 mg/oz. Energy drink companies must however adhere to the federal regulations that have been put in place regarding proper labeling when it comes to caffeine. Those regulations in the U.S. state that caffeine, along with any other ingredient, must be listed on the product label if added to the product as an ingredient. However, the amount of caffeine, or any other ingredient for that matter, does not need to be listed on the label leaving consumers in the dark in regards to actual amount they are consuming. Efforts are being done to change this lack of regulation as discussed in the 5th amino acid assessment workshop, giving priority to developing a safe upper limit for taurine due to the high concentration of taurine in many energy drinks (Munro, 2006). Many countries have addressed this concern and have developed regulatory guidelines for energy drink manufactures to follow. The Australia New Zealand Food Authority (ANZFA) defined a distinct category of beverages called "formulated caffeinated beverages" which must contain no less than 145 mg/l and no more than 320 mg/l of caffeine, including "all caffeine present from whatever source". The European Union has not set an upper limit for caffeine however if the beverage contains greater than 150 mg/l, the product label must read "High Caffeine Content" followed by the amount of caffeine. An initial first step needs to be taken by the FDA in regards to the regulation of energy drinks, which could be as simple as requiring the manufacturer of these products to list the caffeine content as well as supply health warnings if their product contains caffeine in the amount of a specified upper level. An increasingly diverse segment of the population is choosing to consume energy drinks amplifying the importance of implementing a formal regulatory plan for the production and sales of energy drinks.

2. Caffeine

2.1 Sources

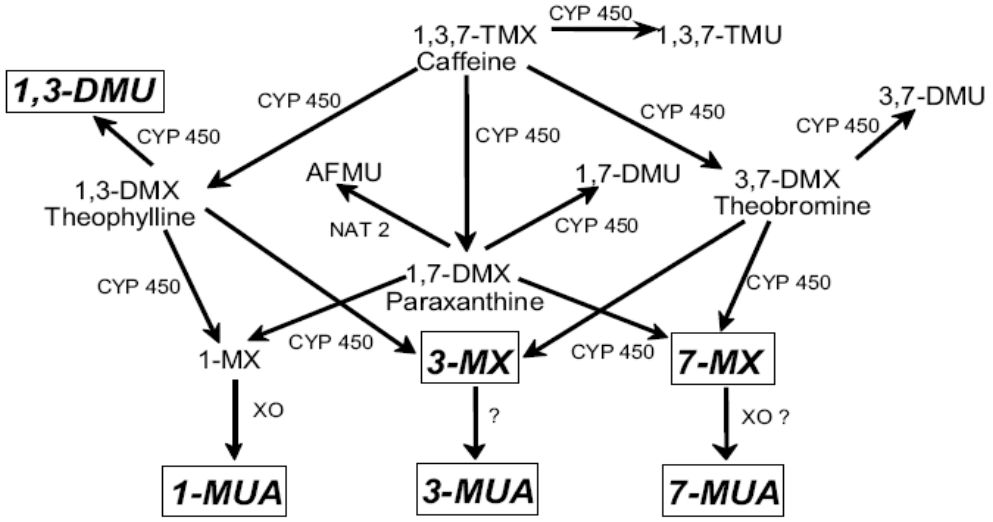
Caffeine is a naturally occurring alkaloid that is found in varying quantities in the beans, leaves, and fruits of more than 60 plants. Some common sources of caffeine are the kola nut (*Cola acuminate*), cacao bean (*Theobroma cacao*), yerba mate (*Ilex paraguariensis*), and guarana berries (*Paullinia cupana)*; however, roasted coffee beans (*Coffea Arabica* and *Coffea robusta*) and tea leaves (*Camelia siniensis*) are the world's primary sources of dietary caffeine (Barone and others, 1996). In the U.S., most of all dietary caffeine consumed is imported in the form of coffee and tea; cocoa, kola nuts and synthetic caffeine account for only a small portion (Bonita and others, 2007; Frary and others, 2008). There is no chemical difference between synthetic caffeine and naturally sourced caffeine. Caffeine is consumed most frequently in beverages such as coffee (71%), soft drinks (16%), and tea (12%) (Beverage Spectrum, 2008). The market for caffeinated beverages has increased in the past decade with the introduction of functional beverages, including the energy drinks category, as well as other beverages such as caffeinated sport drinks, juices and waters (Beverage Spectrum, 2008). In addition to these beverages, caffeine is also found in cocoa, chocolate, and in a variety of medications such as in some pain reliever formulations and in dietary supplements.

2.2 Metabolism

Once ingested, caffeine is rapidly absorbed from the gastrointestinal tract into the bloodstream and becomes metabolized in the liver (Nawrot and others, 2003). Caffeine is extensively metabolized by the liver (99%) to form three major metabolites 3,7-dimethylxanthine,1,7-dimethylxanthine and 1,3-dimethylxanthine showing that 70–

100 mg of caffeine indicating a linear pharmacokinetics (Bonati and others, 1982). For higher doses (250–500 mg), the clearance of caffeine is significantly reduced and its elimination half-life is prolonged, indicating non-linearity (Kaplan and others, 1997). **Figure 4A** shows an overview on how caffeine undergoes demethylation, resulting in paraxanthine (84%), theobromine (12%) and theophylline (4%), with the xanthenes theobromine and theophylline having very similar chemical structures compared to caffeine (**Figure 4B)** (Safranow and Machoy, 2005). These metabolites are then broken down further in the liver by additional demethylations and oxidation to urates with about 3% of those metabolites being caffeine when recovered in the urine (Mandel, 2002). Approximately 90% of the caffeine contained in one cup of coffee is cleared from the stomach within 20 min and peak plasma concentration is reached within approximately 1 to 1.5 h (Chvasta and Cooke, 1971; Nawrot and others, 2003). Once absorbed, caffeine exerts a variety of physiological actions to diverse organs of the body. In doses typically contained in coffee, tea, and soft drinks caffeine's main mechanism of action is to work as an adenosine receptor antagonist in the brain resulting in inhibitory effects to the central nervous system (**Figure 5A** and **5B**) (Dunwiddie and Mansino, 2001; Pettenuzzo and others, 2008). Since caffeine has a similar molecular structure to adenosine**,** with both having a comparable double bond ring structure, caffeine has the potential to occupy adenosine receptor sites, primarily A_1 and A_{2a} (Fisone and others, 2004). The A_1 receptors are located in all parts of the brain with the heaviest concentration in the hippocampus, cerebral and cerebellar cortex and certain thalamic nuclei (Fredholm and others, 1999). The A_{2a} receptors are located in the dopamine rich areas of the brain (Fredholm and others, 1999). After caffeine connects to those receptors an adenosine

A



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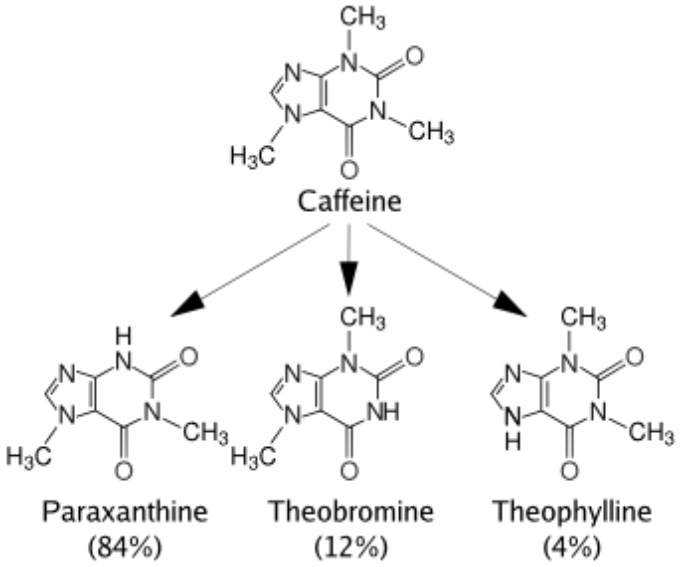
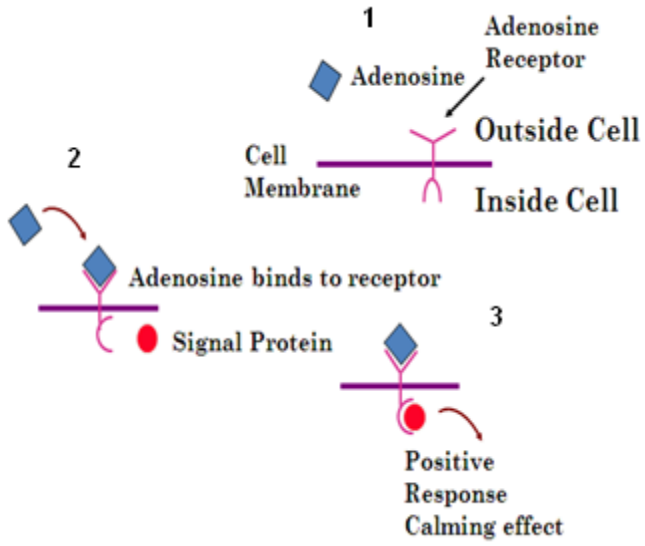


Figure 4. Structure of caffeine and its metabolism (Taken from Safranow and Machoy, 2005). (**A**) Metabolism, (**B**) Chemical structures. Abbreviations: **AFMU**: 5-acetylamino-6-formylamino-3-methyluracil; **CYP 450**: Cytochrome P450; **DMU**: dimethyluric acid**; DMX**: dimethylxanrthines**; MUA**: monomethyluric acid**; MX**: monomethylxanthines**; NAT 2**: N-acetyltransferase 2; **TMU**: trimetylxanthines**; TMX**: trimethylxanthines**; XO**: xanthine oxidoreductase.

A



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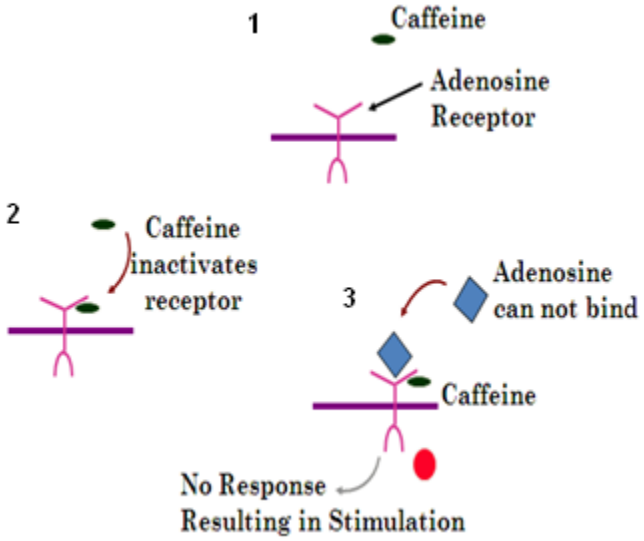


Figure 5. The mechanism of action of caffeine. (**A**) Normal action of adenosine; (**B**) Action of caffeine as an adenosine receptor antagonist.

blockage forms. The blockage of adenosine to the neurons causes the sleep promoting effects of adenosine to stop, resulting in the neurons speeding up (Ferre, 2008).

2.3 Caffeine Consumption and Human Health

Caffeine has been widely studied in a variety of areas regarding human health and performance (Smit and Rogers, 2002). Many studies confirm caffeine's ability to enhance mood and alertness (Kaplan and others, 1997; Lorist and Tops, 2003), exercise performance (Doherty and Smith, 2004), the speed at which information is processed, awareness, attention and reaction time (Cysneiros and others, 2007). Although caffeine is being researched in a variety of areas regarding human health including Parkinson's disease, skin cancer, and diabetes, the focus of this research was caffeine's potential role as an aid in weight loss and weight maintenance. Caffeine consumption, as contained in green tea, has been associated with weight reduction which demonstrates caffeine to be a potential tool for weight management through stimulating thermogenesis and fat oxidation (Kovacs and others, 2004; Westerterp-Plantenga and others, 2005). Additionally, caffeine supplementation has recently been considered as an effective means of weight management (Greenberg and others, 2006; Turk and others, 2009). Energy balance is the main determinant of weight regulation. In this context, research on caffeine has demonstrated its role in increasing metabolic rate, energy expenditure, lipid oxidation, and lipolytic and thermogenic activities; all favorable components in regards to weight management and possible weight loss in humans (Acheson and others, 2004; Ballard and others, 2006; Dallas and others, 2008). Although the role of thermogenic activity in weight control is in the process of being analyzed, multiple studies have shown that caffeine can influence energy balance in humans. In a recent investigation, the 24-h

increased energy expenditure from the caffeine consumption of 300 mg/d was found to be approximately 79 kcal/d, which may appear insignificant; however, it has been found to be sufficient in maintaining weight balance (Rudelle and others, 2007). The American population has an average weight gain of approximately 1 kg/yr, equivalent to 15 kcal/d of excess energy (Hill and others, 2003). It is also known that for 90% of the population, about 50 kcal/d are in excess of their daily energy expenditure (EE), which means that a 50 kcal/d reduction could offset weight gain (Hill and others, 2003). That being said, an increased EE of approximately 79 kcal/d can act as a positive contributor to maintaining, if not losing weight for active individuals (Rudelle and others, 2007). One potential mechanism that could explain the thermogenic effect of caffeine is through the inhibition of cyclic AMP- phosphodiesterase (PDE) and the antagonizing adenosine receptors which negatively affect increased norepinephrine (NE) release (Westerterp-Plantenga and others, 2006). The consumption of caffeine would consequently result in increased cyclic AMP, causing a heightened level of NE allowing the adrenoceptors to be continually stimulated (Dulloo and others, 1999; Dulloo and others, 2000). However, the effect of caffeine on weight reduction should be interpreted with caution since for example, green tea drinking is effective only in individuals with originally low caffeine intake (< 300 mg/day) (Kovacs and others, 2004). Furthermore, in a long term observational study, caffeine intake was only associated with less weight gain (Lopez-Garcia and others, 2006). The use of caffeine to manage weight should consider the calorie contribution of the vehicle that contains caffeine and the potential negative effects of caffeine.

3. Obesity and Weight Management

Obesity is considered an epidemic of modern society and its prevalence

ontinues to increase, illustrating the importance for continued research in the area of obesity and weight management (Abelson and Kennedy, 2004). The ultimate cause of obesity is a chronic imbalance between the kilocalories consumed and the kilocalories expended resulting in an increase in adipose tissue due to an increase in both adipose cell number and size (Stunkard, 1996). In order to lose weight a negative kilocalorie balance needs to result and can be achieved by either decreasing intake or increasing expenditure. **Figure 6** outlines adipogenesis, which is the process by which new fat cells develop from adipocyte precursor cells called preadipocytes which into mature adipocytes (Avram, 2007). The amount of adipose tissue can be regulated through the inhibition of adipogenesis and through reduced accumulation of fat (Rayalam and others, 2008). Adipogenesis involves two major events the recruitment and proliferation of preadipocytes followed by their subsequent differentiation into mature fat cells. Fat accumulation in adipose tissue can be decreased by reducing lipid uptake by adipocytes through the suppression of lipoprotein lipase (LPL) or reducing lipid synthesis through inhibiting fatty acid synthase (FAS) among other mechanisms (Jing-Jing and others, 2008; Wang and Eckel, 2009). Increased research in the area of bioactive compounds and their potential role for the improvement of long term weight maintenance is needed. Weight loss is increasingly recognized to have major health benefits for overweight individuals as well as increase the life expectancy of those suffering from obesity-related health complications (Goldstein, 1992; Westerterp-Plantenga and others, 2005; Rayman and others, 2008). It has been found that modest weight loss of just 5% to 10% of initial body weight can lead to beneficial health effects (Goldstein 1992; Wing and others, 1992; van Gaal and others, 1997). Caffeine has been shown to have anti-obesity effects through

the suppression of body weight gain and adipose tissue formation (Diepvens and others, 2007; Huang and others, 2009). Additionally, caffeine has been reported to suppress body weight gain through stimulating thermogenesis, extending sympathetic stimulation, suppressing food intake and reducing adipose tissue mass (Dulloo and others, 1999, 2000; Hasegawa and Mori, 2000; Zheng and others, 2004; Kazuo and others, 2005; Kobayashi-Hattori and others, 2005; Lopez-Garcia and others, 2006; Rashti and others, 2009; Tanka and others, 2009). Caffeine consumption could be an effective tool to increase the success rate of long-term weight maintenance and possible weight loss in humans.

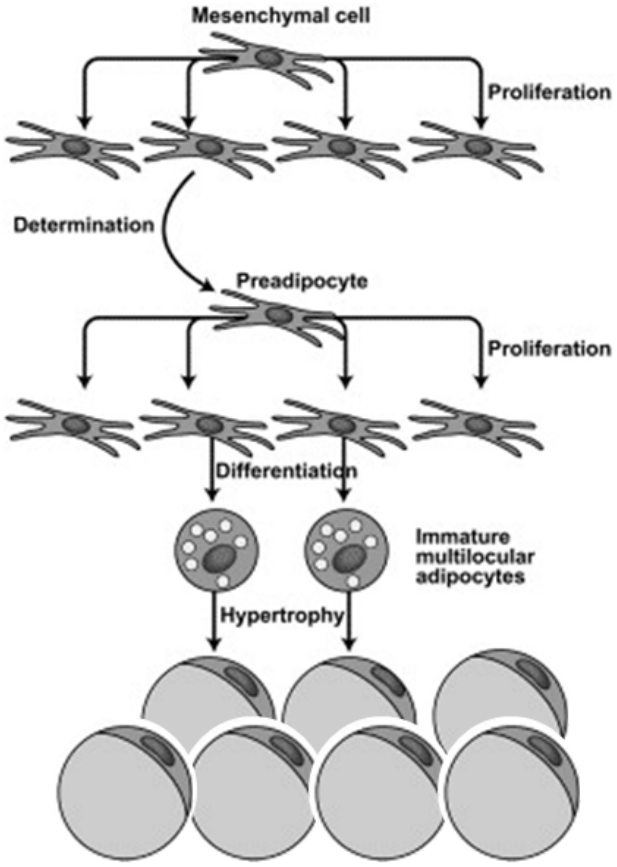


Figure 6. Adipogenesis: The proliferation and differentiation process of preadipocytes into mature adipocytes. (Adapted from Avram and others, 2007)

III. RESEARCH SIGNIFICANCE

Unhealthy weight gain is a global health concern due to its many well-recognized health implications. With the incidence of obesity significantly increasing worldwide, research in the area of bioactive food components geared towards weight management would be very beneficial. Since the energy drink market has seen impressive growth over the past decade and is expected to continue, it is a great avenue to develop and expand the health functionality of those beverages. Given that synthetic caffeine is a prominent ingredient in energy drinks, and is also associated with body weight regulation and decreased lipid accumulation, makes it an ideal compound for further investigation. Green coffee byproducts and the caffeine extracted from Yerba mate tea (matein) could be as effective as synthetic caffeine to inhibit *in vitro* lipid accumulation in adipocytes, making them a potential natural source, as well as an economical and environmentally sound alternative to synthetic caffeine for the incorporation into energy drink formulations.

IV. HYPOTHESIS AND OBJECTIVES

1. Hypothesis

    - Yerba mate caffeine (matein) and caffeine from green coffee byproducts are as effective as synthetic caffeine in inhibiting *in vitro* adipogenesis and lipid accumulation and can be incorporated into energy drink formulations.

2. Main Objective

    - Develop a natural caffeine blend to replace synthetic caffeine in energy drinks with equivalent effectiveness to inhibit lipid accumulation.

3. Specific Objectives

    - To determine caffeine concentration of matein and green coffee byproducts
    - To determine the lipid reduction potential of matein and green coffee byproducts in comparison to synthetic caffeine
    - To determine if a synergistic lipid inhibitory effect is present when a blend of matein and green coffee byproducts are used
    - To quantify the antioxidant capacity and polyphenol concentration in matein and green coffee byproducts
    - To develop a prototype energy drink formulation incorporating natural sources of caffeine and antioxidants

V. MATERIALS AND METHODS

1. Materials

1.1 Chemical and Biological Materials

Synthetic caffeine (SC) (99%), Isobutylmethylxanthine (IBMX), dexamethasone (DEX), insulin, 100 mM sodium pyruvate solution, penicillin (1000 U/ml), streptomycin (1000 U/mL), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-azobis 2-amidinopropane dihydrochloride (AAPH), formic acid (>95%), gallic acid (100%), HPLC grade methanol, Oil Red O dye, Orlistat, C75, butylated hydroxy toluene (BHT), epigallocatechin gallate (EGCG; ≥ 95% purity), and citric acid (99.5% purity) used in the prototype beverage formulation were purchased from Sigma-Aldrich (St. Louis, MO). TIC Pretestol Ticalose CMC 2500 Fine Powder was the suspending agent used in the prototype beverage formulation and was provided by TIC Gums (Belcamp, MD). Swiss albino mouse 3T3-L1 fibroblasts and Dulbecco's modified Eagle's medium (DMEM) were purchased from American Type Culture Collection (Manassas, VA). Cell Titer 96 Aqueous One Solution was purchased from Promega (Madison, WI) to determine cell viability. Fetal bovine serum (FBS), calf bovine serum (CBS), Dulbecco's phosphate buffered saline, diethylpyrocarbonate (DEPC) treated water, SYBR Green PCR Master Mix and trypsin-EDTA were purchased from Invitrogen (Grand Island, NY). Fluorescein (3',6' dihydroxyspiro [isonezofuran-1[3H] 9'[9H]-xanthen]-3-one), HPLC grade water (99%), and Folin-Ciocalteu's phenol reagent (2 N) was purchased from Fisher Scientific (Hanover Park, IL). RNeasy Lipid Tissue Mini Kit was purchased from Qiagen (Valencia, CA) to extract mRNA from adipocytes. Primers were purchased from Integrated DNA

Technologies (Coralville, IA).

1.2 Natural Caffeine Sources and South American Berries

The material which was studied included four green coffee byproducts from coffee processing (fractions GC1, GC2, GC3 and GC4) provided by a coffee manufacturing facility in Houston, Texas and the mate caffeine was extracted from Organic Guayaki Yerba Mate Tea, referred here as matein (MT) (**Figure 7**).

Figure 7. Visual representation of the four green coffee byproducts (GC1, GC2, GC3 and GC4), matein (MT), and Yerba mate tea.

In addition to the natural caffeine blend, a variety of South American berries, including acaí, maqui, and mortiño berries were incorporated into a beverage formulation in order to increase consumer product appeal as well as to enhance the overall health functionality of the beverage. Acaí *(Euterpe oleracea)* (**Figure 8A)**, is a berry of the palm family, which has a long history of use due to its potential health benefits associated with the berries high antioxidant capacity and phytochemical composition (Schauss and others, 2006). The freeze dried acaí berry was donated by the Liotécnica Company located in Embu, São Paulo, Brazil. Maqui (*Aristotelia chilensis*) (**Figure 8B)**, is a shrub growing berry which has also a long history of use primarily in folk medicine to treat aliments such as sore throats, inflammation, diarrhea, lesions, and migraines (Schrekinger and others, 2010). Maqui has been reported to have high concentrations of polyphenols, especially anthocyanins (Escribano-Bailon and others, 1990). The maqui berries were collected in January 2009 from the Entrelagos region in Chile and were cleaned by removing leaves, stems and damaged berries. The whole berries were then freeze-dried, sealed in plastic bags, and shipped to our laboratory. The mortiño berry (*Vaccinium floribundum*) (**Figure 8C**) is a shrub growing berry that is widely consumed in Ecuador as fresh fruit or as processed products and is known for their high phenolic content (Shreckinger and others, 2010). Local communities have used the mortiño berry to treat inflammation and diabetes which can be attributed to their high phenolic content. The mortiño berry is a natural product produced by the Simiatug Samai which is actually a commercially available product that was purchased from a local market in Quito, Ecuador. This commercial powder was prepared by dehydrating the fresh berry at low temperatures (< 45 ^{o}C) and high ventilation.

A

Acaí berry

Acaí Palm

Freeze dried acaí

B

Maqui berry

Maqui plant

Freeze dried maqui

C

Mortiño berry

Mortiño plant

Commercial mortiño

Figure 8. Visual representation of berries native to South America that were incorporated into the beverage formulation, (**A**) açaí *(Euterpe oleracea*), (**B**) maqui (*Aristotelia chilensis*), and (**C**) mortiño (*Vaccinium floribundum*); (Schrekinger and others, 2010).

1.3 Commercial Energy Drinks

A variety of commercial products including 16 energy drinks and 15 ready to drink tea-based energy drinks, were purchased directly from several markets in Champaign, IL. Product names are listed in the appendix.

2. Methods

2.1 Preparation of matein, green coffee byproducts and yerba mate tea

Matein (MT) was obtained by supercritical CO_2 extraction of mate tea leaves. The supercritical extraction apparatus was provided by the U.S. Department of Agriculture (Peoria, IL). MT was extracted from organic mate tea using a water saturated CO_2 supercritical extraction. The temperature for the extraction vessel was 70˚C with a pressure of 398 bar. The flow rate was 466 ml/min of liquid CO_2. A total of 1.5 kg of mate tea was placed in the supercritical CO_2 extractor, and it took approximately 8 h for the entire procedure to be completed (Assis and others, 2006). The final product was freeze dried.

The decaffeination process of raw green coffee at the processing plant resulted in 4 separate byproducts (GC1, GC2, GC3 and GC4), supplied by the coffee manufacturing facility, each containing different concentrations of residual caffeine. Green coffee byproduct 1 (GC1) was produced by the CO_2 supercritical extraction technique; however, the second green coffee byproduct (GC2) was derived using water alcohol extraction. Green coffee 3 (GC3) was a dried extract from a slurry known as liquid mother, a concentrated liquid extract effluent of coffee processing and the fourth byproduct (GC4) consisted mainly of the fiber from the green coffee grain. The yerba mate tea brewing process was carried out following the protocol described by Chandra

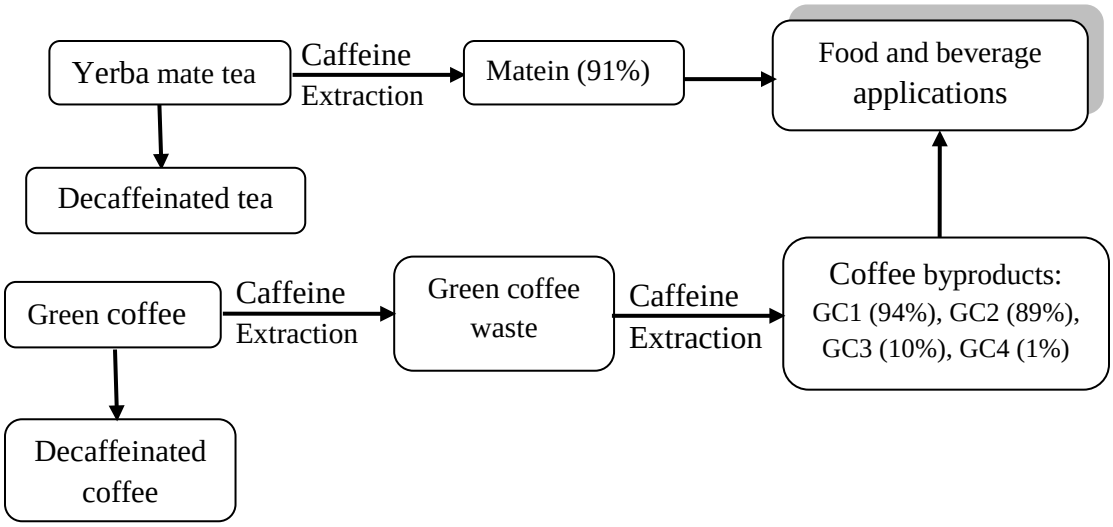


Figure 9. Schematic of the production of green coffee byproducts and matein.

and Gonzalez de Mejia (2004). Briefly mate tea (2.7 g) was mixed with 250 ml of boiling water at 98˚C and held/stirred for 10 min. The tea extract was filtered using Whatman paper #2 and lyophilized in a FreezeZone freeze dry system (Kansas City, MO) and stored at -20˚C until the incorporation into the mate tea based energy drink prototype.

2.2 Chemical Analysis

2.2.1 Caffeine Quantification

The caffeine concentration was quantified for the four green coffee byproducts (GC1, GC2, GC3 and GC4) and MT using high performance liquid chromatography (HPLC). The quantification was carried out on an 1100 HPLC (Agilent Technologies, Santa Clara, CA) using a reversed phase Supelcosil-LC-18 column (250mm x 4.6mm x 5µm) (Supelco, Bellefonte, PA). Samples were dissolved at a concentration of 2 mg/ml and diluted down to a concentration of 0.1 mg/ml in 100% methanol and filtered through

0.22 μm filters. Caffeine standards were prepared using commercially available pure caffeine (99.9%) at concentrations of 0.125, 0.25, 0.5, 0.75, 1.25 and 1.5 μg/ml and then filtered. The mobile phase consisted of 20% methanol, 0.3% formic acid and 79.7% water (A) and 0.3% formic acid in methanol (B). The flow rate was held constant at 0.5 ml/min with a step-wise gradient of 0%, 48% and 0% of solvent B at 52, 5 and 3 min, respectively. The diode array detector was set for outputs of 280 and 330 nm. The injection volume was 50 μL for the samples and 20 μL for the caffeine standards. Agilent's Chemstation software was used for both protocol control and data processing. Caffeine concentrations in mg/ml was calculated from the caffeine standard curve ($y = 5216.4x - 29.02$, $R^2 = 0.99$).

2.2.2 Elemental Mineral Analysis

Elemental mineral analysis was performed after first digesting the samples with HNO_3 in a PerkinElmer/Anton Paar Multiwave 3000 microwave digester, using a high pressure rotor. The samples coming out of the digester were diluted to 50 ml. The samples were analyzed using a PerkinElmer Elan DRCe Inductively Coupled Plasma (ICP) MS instrument (Norwalk, CT), in Total Quant (TQ) mode. This method was reinforced through the use of a series of 50 ppb standards. The mineral concentration was expressed in ppm.

2.2.3 Determination of Total Polyphenol Content

Total phenolic content was measured using the Folin-Ciocalteu method, adapted to a micro-assay, from the method described by Chandra and de Mejia (2004). Briefly, samples were prepared for the green coffee byproducts and matein by dissolving the powder in water and filtering with a 0.22 μM syringe filter; final concentration was 1

mg/ml. To a 96-well flat bottom plate (Fisher 12-565-501), 50 µL of 1 N Folin-Ciocalteu's phenol reagent was added, then 50 µL of either sample, standard or blank were added; this mixture was allowed to stand for 5 min before the addition of 100 µL 20% $NaCO_3$. The solution is then allowed to stand for 10 min and under the alkaline environment a reduction reaction takes places causing a shift in absorbance from yellow to blue, which is read at 690 nm in a fluorescent FLx800tbi Synergy 2 multi-well plate reader, (BioTek, Winooski, VT). Gallic acid (GA) was used as a standard; with final concentrations of 10, 20, 30, 40, 50, 75 and 100 µg/ml. Results were expressed as µg equivalents (eq.) GA, using the standard curve $y = 0.027x - 0.106$, $R^2 = 0.99$.

2.3 Antioxidant Capacity

The ORAC assay was adapted from published methods (Davalos and others, 2004; Prior and others, 2003). Fluorescein and AAPH solutions were prepared using 75 mM phosphate buffer, pH 7.4. In triplicate (20 µL), samples GC1, GC2, GC3, GC4, MT, SC, EGCG, and BHT at concentrations ranging from 250 µM to 1000 µM, Trolox standard dissolved in 75 mM phosphate buffer at concentrations ranging from 4 µM to 120 µM, or 75 mM phosphate buffer blank were added to a 96-well black walled plate. After which, 120 µL Fluorescein was added and incubated for 15 min at 37˚C. After incubation 60 µL AAPH was added to a final concentration of 153 mM. The fluorescence was read immediately in a fluorescent FLx800tbi Synergy 2 multi-well plate reader, (BioTek, Winooski, VT), at 37˚C, sensitivity 60, read every 2 min for 120 min with excitation 485 and emission 528 nm. Fluorescein reacts with the free radicals generated by the AAPH to produce a non-fluorescent product. Loss of fluorescence is easured over time and the area under the curve is calculated with the following equation:

$$AUC = 0.5 + f_1/f_0 + f_i/f_0 + \ldots + 0.5(f_n/f_0)$$

$$\text{Net AUC} = AUC_{sample} - AUC_{blank}$$

Where AUC is the area under the curve, f_1 is fluorescence of first reading (2min), f_0 is fluorescence of reading time zero and f_n fluorescence readings. Results were expressed as Trolox μM eq., using the standard curve y = 0.167 + 2.54, $R^2 = 0.99$.

2.4 Lipid Metabolism in 3T3-L1 Adipocytes

2.4.1 Cell culture and treatments

Figure 10 illustrates the process in which the 3T3-L1 preadipocytes underwent to develop into mature adipocytes. First the 3T3-L1 preadipocytes were seeded at 3 x 10^4 cells/cm^2 in 24-well plates and cultured in DMEM containing 10 mM sodium pyruvate, 100 U/mL penicillin, 100 U/mL streptomycin and 10% FBS (FBS/DMEM). The preadipocyte differentiation was induced 3 days after reaching 100% confluency. The cells were stimulated with FBS/DMEM containing 167 nM insulin, 0.5 M IBMX and 1 M DEX for 2 days, followed by culturing with FBS/DMEM for an additional 4 days, at which time 90% of cells were mature adipocytes with accumulated fat droplets. Mature adipocytes were treated separately at a non-cytotoxic concentration of 100 μM with SC, MT, GC1, GC2, GC3, GC4, MT/GC1 blend, EGCG, C75, and Orlistat with all samples dissolved in 0.2% dimethyl sulfoxide (DMSO), with the highest final concentration of DMSO used being 0.2%. Furthermore, the cells were treated throughout the differentiation process on days 3, 5, 7 and 9 with 100 μM SC, MT, GC1, and a 50/50 blend of MT/GC1. All treatments were compared to a negative control (untreated cells) and several positive controls including EGCG, Orlistat, and C75. Adipocytes were incubated at 37 ˚C in a 5% CO_2 atmosphere for 48 h before the lipid

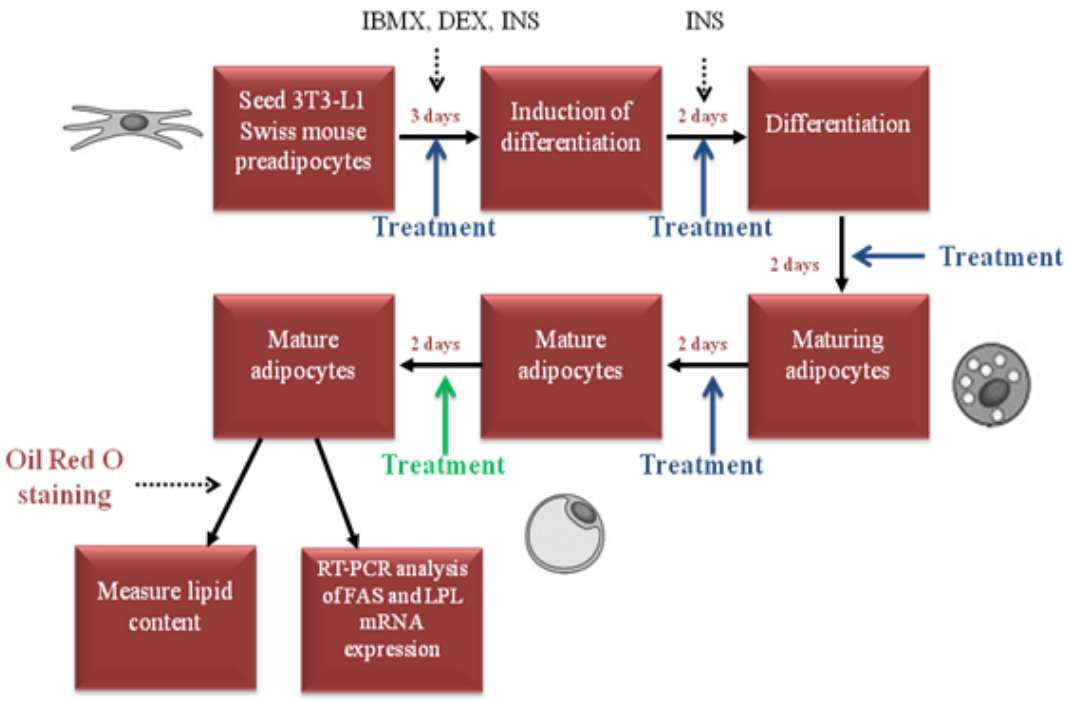


Figure 10. Cell culture and treatment of 3T3-L1 adipocytes. **Abbreviations: IBMX**: 3-isobutyl-1 methylxanthine; **DEX**: d examethasone; **INS**: insulin.

quantification was determined using the Oil Red O staining assay.

2.4.2 3T3-L1 adipocytes cell viability assay

The 3T3-L1 preadipocytes were seeded at 1.5 x 10^4 cells/cm^2 in 96-well flat-bottom cell culture plates where they underwent differentiation and then were treated as indicated above. Additionally, preadipocytes were also treated throughout the differentiation on days 3, 5, 7, and 9 to determine cytotoxic effects. The CellTiter 96Aqueous One Solution was used to determine the number of viable cells according to the manufacturer's manual (Promega, Madison, WI). Briefly, 20 µL of the CellTiter AQuaous One Solution was added to 100 µL of medium containing wells with cells, and then the plate was incubated in a 5% CO_2 incubator at 37 ˚C. After 2 h, absorbance was

measured at 515 nm with a 96-well plate reader (Biotek Instruments, Winooski, VT). Cell viability percentages were calculated using the following equation:

$(A_{\text{treatment, 515nm}}/A_{\text{control, 515nm}}) * 100 = \%$ cell viability.

2.4.3 Lipid quantification in 3T3-L1 adipocytes using Oil Red O staining

Intracellular lipid accumulation was determined by Oil Red O staining during adipocyte differentiation. Throughout differentiation, adipocytes accumulate fat droplets and become spherical in shape. Briefly, treated adipocytes were washed with Dulbecco's phosphate buffered saline (DPBS) and fixed with 10% formalin (in DPBS) in 24-well plates for 1 h. Then, cells were washed with 60% isopropanol and allowed to air dry. The Oil Red O stock solution (0.1 g Oil Red O in 50 ml of 40% isopropanol) was added to lipid droplets for 1 h. After Oil red O lipid staining, cells were washed four times with water to remove any excess stain and left to air dry. Oil Red O dye was eluted by adding 100% isopropanol then incubating at room temperature for 10 min. OD_{510nm} of eluted isopropanol was measured using a FLx800tbi Synergy 2 multi-well plate reader (BioTek, Winooski, VT). Inhibition percentages of lipid accumulation were calculated using the following equation:

$((A_{\text{control, 510nm}} - A_{\text{treatment, 510nm}})/A_{\text{control, 510nm}}) * 100 = \%$ inhibition of lipid content.

2.4.4 RT-PCR analysis

Total mRNA was extracted from matured 3T3-L1 adipocytes treated with 100 μM SC, MT, GC1, MT/GC1 blend, EGCG, Orlistat and C75 using RNA easy mini-kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. RNA quality and concentration were determined by agarose gel electrophoresis and nanodrop spectrophotometry (ND-100 NanoDrop Tech, Wilmington, DE). RNA expression of

target genes was measured using real-time quantitative PCR with SYBR Green fluorescence dye (Applied Biosystems, Foster City, CA). Briefly, 2 µg purified RNA was reverse transcribed into complementary DNA. Published primer sequences were used for LPL and FAS with 18S ribosomal RNA used as an internal control (Gonzales and Orlando, 2007; Koo and others, 2008). The following primer sequences were used:

LPL: forward 5'- CTGCTGGCGTAGCAGGAAGT-3'
reverse 5'- GCTGGAAAGTGCCTCCATTG-3'

FAS: forward 5'- TCGGCGAGTCTATGCCACTATT-3'
reverse 5'- ACAGAGAACGGATGAGTTGTTCCT-3'

18S: forward 5'- GATCCATTGGAGGGCAAGTCT-3'
reverse 5'- AACTGCAGCAACTTTAATATACGCTATT-3'

The plate was centrifuged with CR-422 centrifuge machine (Jouan, Inc., Frederick County, VA) and read with Taqman 7900 HT Real-time PCR System machine (Applied Biosystems, Foster City, CA). The mRNA abundance relative to 18S was determined using comparative critical threshold method according to manufacturer's instructions.

2.5 Statistical Analysis

Statistical analysis was performed using SAS version 3.1 (SAS Inst. Inc., Cary, NC). The caffeine concentration, AC, TPC, and the *in vitro* lipid accumulation inhibition were evaluated by analysis of variance (ANOVA) using the proc GLM function for all treatments. Statistical significance was identified with an alpha of 0.05 and means comparison were made based on *P*-values obtained using Tukey's HSD test. All analyses were based on at least 2 independent trials with 3 replicates per trial.

VI. RESULTS AND DISCUSSION

1. Chemical Analysis

1.1 Determination of Caffeine Concentration

Figure 11 depicts the caffeine concentration (%) present in MT and GC byproducts compared to SC (99.9%). MT, GC1, and GC2 were found to have the highest caffeine concentrations with values of 91, 94, and 89% caffeine respectively ($p < 0.05$). GC3 and GC4 contained far less caffeine than expected, with values of 10% and 1% caffeine, respectively. GC3 was a dried extract of liquid mother, a sludge that comes off of the decaffeination process of green coffee and GC4 is the byproduct from the outer shell of the green coffee bean containing over 20% fiber. These results suggest that MT,

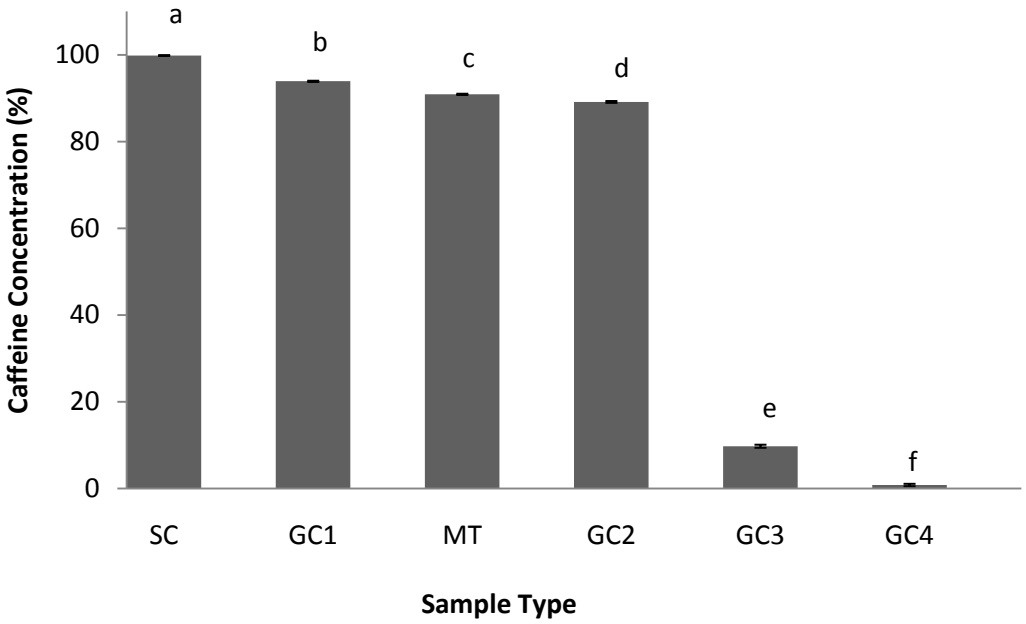


Figure 11. Caffeine concentration (%) of MT, GC1, GC2, GC3, and GC4 compared to SC. Bars represent means ± standard deviation based on data from three independent experiments. Different letters indicate significant difference ($p < 0.05$).

GC1, and GC2 would be excellent natural alternatives to SC in food and beverage applications.

1.2 Elemental Mineral Analysis

Table 1 shows the concentration of several nutritionally essential minerals that are present in MT and GC1. MT and GC1 contained similar iron concentrations with reported values of 30.5 and 35.5 ppm, respectively. GC1 was found to have higher concentrations of calcium, magnesium, and potassium, whereas MT was found to have higher concentrations of zinc. It has been reported that the consumption of mate tea results in a more balanced and sustained energizing feeling compared to other caffeinated beverages. This is thought to be due to a synergy between the caffeine and other chemical constituents present in mate tea, including their mineral contents. Unfortunately there has been no documented research that verifies this hypothesis.

Table 1. Mineral concentration present in MT and GC1.

Mineral	Mineral Concentration (ppm)	
	MT	**GC1**
Calcium	19.4	105
Chlorine	2620.4	1272.7
Copper	1.8	0.4
Iron	30.5	35.5
Magnesium	8.9	25.7
Manganese	0.3	0.5
Potassium	30.1	280
Sodium	59.1	1850.4
Zinc	40.1	0.3

ppm = parts per million (1 mg/kg = 1 ppm)

1.3 Total Polyphenol Content

Figure 12 illustrates the total polyphenol content (TPC) of MT and the GC byproducts determined by the Folin-Ciocalteu method, using gallic acid (GA) as the standard. SC was used as a negative control since it does not contain any phenolic compounds, however, due to its aromatic structure showed a background reaction with the Folin-Ciocalteu reagent. GC3 (153.5 µg GA eq./mg) and GC4 (64.3 µg GA eq./mg) resulted in the highest TPC compared to GC1, GC2, and MT. These results were expected since GC3 and GC4 were found to have very low caffeine concentrations allowing for other components such as polyphenols to be more concentrated. For a comparison, the TPC of mate tea (2.7 g in 250 ml), known for its numerous active phytochemicals among them being polyphenols, was also analyzed and resulted with

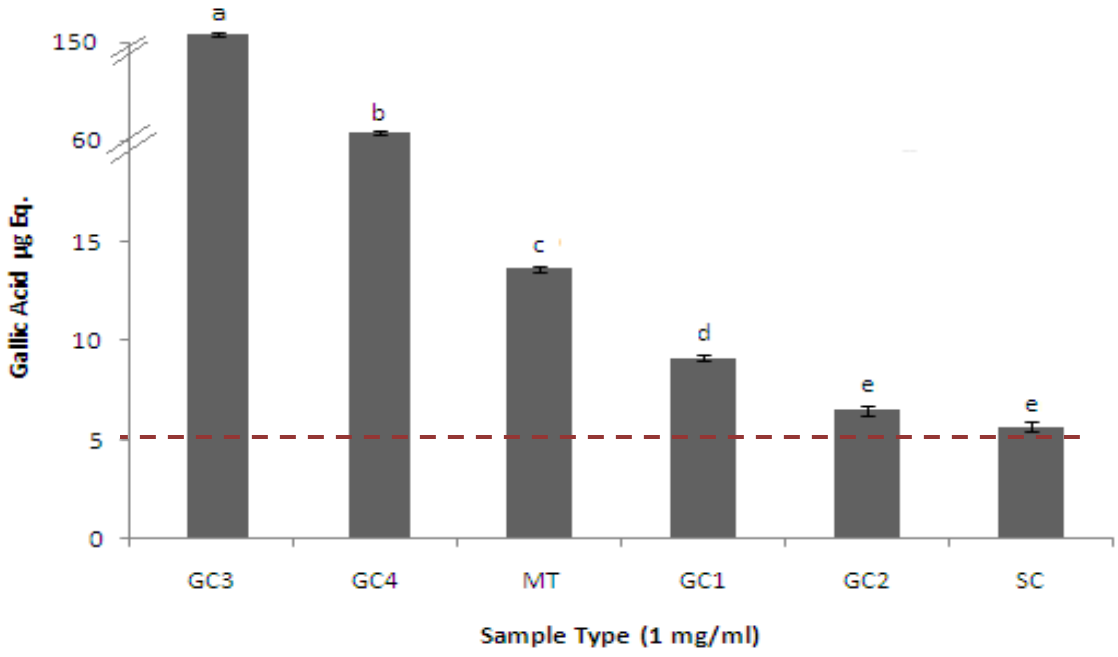


Figure 12. Total polyphenol concentration GC byproducts, MT, and SC expressed as µg equivalents of gallic acid.

213.6 µg GA eq./mg. Among the samples which are comprised primarily of caffeine, MT (13.7 µg GA eq./mg) resulted with the highest TPC followed by GC1 (9.1 µg GA eq./mg), and lastly GC2 (6.5 µg GA eq./mg).

2. Antioxidant Capacity

MT and GC byproducts are both known to contain high amounts of substances with health beneficial properties (Heck and de Mejia, 2007; Ramalakshmi and others, 2009). MT, GC byproducts, and SC were analyzed for their antioxidant content using the oxygen radical absorbance capacity (ORAC) assay, a widely used and preferred method due to its biological relevance to the *in vivo* antioxidant effectiveness (Prior and others, 2003; Davalos and others, 2004). The ORAC method uses the peroxyl radical which is the most common free radical in the human body (Mermelstein, 2008). **Figure 13A** shows the AC of GC3 and GC4 compared to EGCG, a natural compound widely reported for its extremely high AC (Seerman and others, 2006). GC3 and GC4 were found to have a significantly lower AC compared to EGCG, however, still containing a substantial amount of antioxidants. The AC of 1000 µM GC3 was 1550 ± 19 µM Trolox Eq. and GC4 had an AC of 553 ± 7 µM Trolox Eq. Those byproducts contain minimal caffeine, however, could be utilized as a potent antioxidant contributor to a food product. **Figure 13B** depicts the AC of MT, GC1, GC2, MT/GC1 blend, and SC all compared to BHT a well known synthetic antioxidant. MT (77 ± 3 µM Trolox eq.) was found to have similar AC as compared to BHT (71 ± 7 µM Trolox eq.), whereas GC1 had a lower AC of 34 ± 3 µM Trolox eq. in an equimolar basis. Although MT had a higher AC than GC1, when in combination the AC of 71 ± 0.3 µM was similar to MT alone. SC was found to have no AC which is important because the replacement of SC with a blend of GC1 and MT

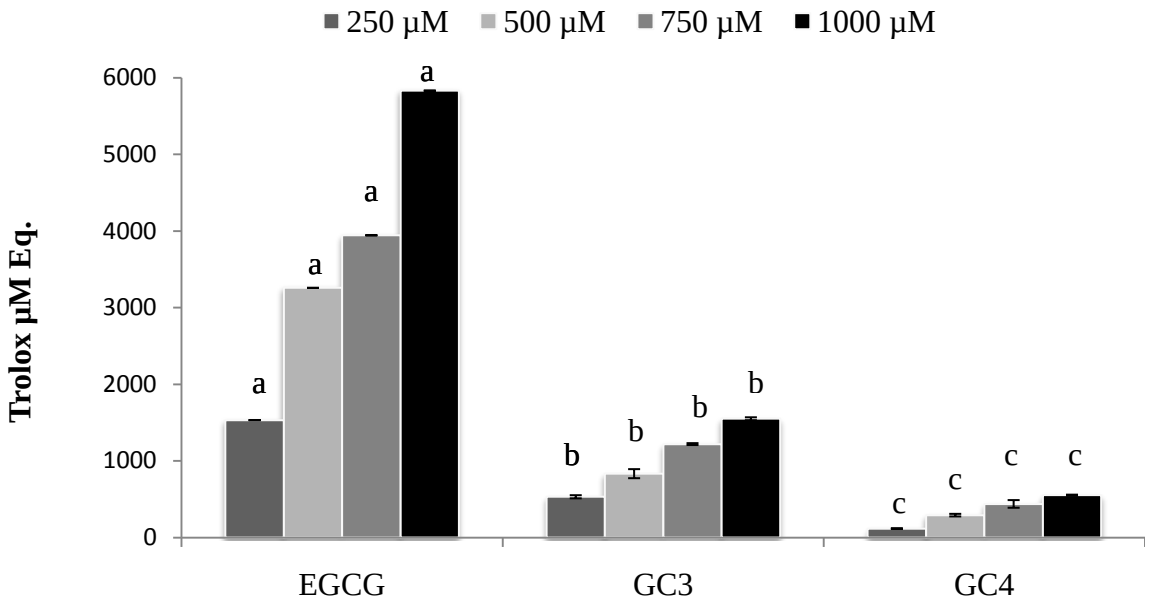
would increase the health functionality of food products while at the same time providing similar amounts of caffeine. Compounds high in antioxidants are important because they help protect cells in the body from the damaging effects of free radicals known to damage proteins, lipids, and DNA, reducing the risk of diseases such as cancer and coronary heart disease (Awika and others, 2003; Miller and others, 2006). These findings are promising in regards to the potential of MT and the GC byproducts being incorporated into beverage formulations as an alternative to SC. In addition to the energy enhancing substances in energy drinks, mainly caffeine, many also contain a variety of health-promoting constituents, including antioxidants and polyphenols which are contributed by the various fruit or tea extracts that makeup those beverage formulations (Heckman and others, 2010). It was found that yerba mate tea-based energy drinks contained 100-fold higher amounts of antioxidants and polyphenols compared to the mainstream non tea-based energy drinks and the traditional brewed yerba mate tea (Heckman and others, 2010). The natural MT and GC byproducts would provide the desired stimulatory effect in addition to contributing to the overall health functionality of a food beverage product.

3. Cell Culture

3.1 Cell viability of 3T3-L1 adipocytes

No effect on the viability of 3T3-L1 adipocytes was observed with any of the concentrations of MT, GC byproducts, SC, EGCG, C75, and Orlistat used in this study, indicating no cellular toxicity up to 200 μM for MT and the GC byproducts and 100 μM for SC, EGCG, C75, and Orlistat.

A



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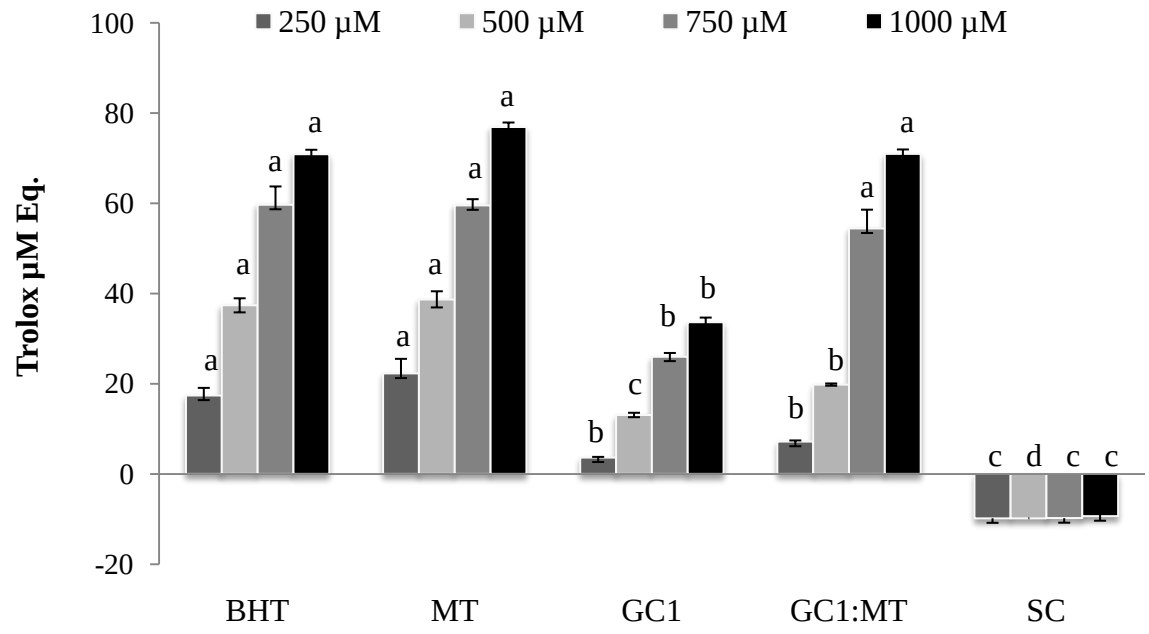


Figure 13. Antioxidant Capacity of MT, GC1, GC2, GC3, GC4, and SC. (**A**) Antioxidant capacity of GC3 and GC4 compared to EGCG; (**B)** Antioxidant capacity of GC1, GC2, MT, and SC compared to BHT. Bars represent means ± standard deviation based on data from three independent experiments. Different letters indicate significant difference among the samples at the same concentration.

3.2 Inhibitory effect of MT and GC byproducts on lipid accumulation in 3T3-L1 adipocytes

Figure 14 represents the percent inhibition of lipid accumulation by Oil Red O staining in completely differentiated 3T3-L1 mature adipocytes after 48 h of treatment with 100 µM MT, GC1, GC2, GC3, GC4, and SC relative to a negative control (untreated cells). The cells were also treated with several positive controls including 100 µM C75 and Orlistat, two well recognized anti-obesity drugs, as well as with100 µM EGCG, the most potent all natural anti-adipogenic compound (Kriedal and others, 2007; Moon and others, 2007; Mera and others, 2009; Thielecke and Boschmann, 2009). **Figure 14A** shows that MT and GC1 had similar lipid inhibitory effect as SC, with MT showing the strongest effect of 18.4 ± 1.1%. Furthermore, MT, GC1, and SC also had similar lipid reduction as compared to Orlistat (17.9 ± 1.1%) and EGCG (17.5 ± 0.6%). GC2 showed significantly less inhibition with a 10.6 ± 1.2% reduction and GC3 and GC4 had 3.6 ± 0.4 and 2.0 ± 1.2%, respectively.

The inhibitory effect of these compounds on lipid accumulation in adipocytes was found to be highly correlated to their caffeine concentration ($y = 5.979x - 4.073$, $R^2 = 0.88$); with MT and GC1 having the highest caffeine concentration of 90.8 and 93.8% as well as the highest lipid reduction. These results are in agreement with previous research that concluded that caffeine suppressed the intracellular lipid accumulation after complete differentiation of 3T3-L1 adipocytes (Nakabayashi and others, 2008). Additional studies have shown caffeine to exude anti-obesity properties through the reduction of adipose tissue weight in experimental animals (Bukowiecki and others, 1983; Michna and others, 2003; Zheng and others, 2004). Moreover, human studies have also shown caffeine to stimulate thermogenesis and the rate of fat oxidation (Dulloo and others, 1989; Astrup

and others, 1990; Bracco and others, 1995; Rumpler and others, 2001). Caffeine supplementation has also been recently considered as an effective means of weight management (Greenberg and others, 2006; Turk and others, 2009). **Figure 14B** depicts that an increased lipid inhibitory effect was present among GC1, MT, and SC after treatment throughout the differentiation process. MT and GC1 resulted in an elevated inhibitory effect, with MT having 22.8 ± 2.2% and GC1 with 26.8 ± 1.8%. GC1 had an 8.6% and MT a 4.4% increased in lipid reduction compared to single treatment of mature adipocytes 48 h before Oil Red O staining. GC1 was found to have similar lipid reduction compared to SC (29.3 ± 0.5%). A significantly greater lipid reduction was seen among C75 (68.7 ± 1.0%), Orlistat (54.7 ± 1.7%), and EGCG (49.9 ± 3.5%) with all of their values significantly higher than MT, GC1, and SC ($p < 0.05$). Previous research contradicts these findings, indicating that caffeine does not inhibit the differentiation of 3T3-L1 pre-adipocytes to mature adipocytes (Nakabayashi and others, 2008). **Figure 15** shows a visual of the Oil Red O stained adipocytes when treated at maturity, (**A**) control (untreated cells) compared to the 3T3-L1 adipocytes treated with (**B**) 100 µM MT which resulted in an 18.4% lipid reduction in both size and number, (**C**) 100 µM GC1 which showed a 17.1% reduction, and (**D**) 100 µM GC1 and MT blend with a 19.7% reduction. **Figure 16A** shows GC1 and MT in combination suppressed lipid accumulation in 3T3-L1 cells in a dose dependent manner; however it did not show a synergistic, but rather, an additive effect. The 10 µM 50/50 blend of MT and GC1 followed the same trend as the individual ingredients resulting in a more effective lipid inhibitory effect when treated throughout differentiation. However, 50 µM and 100 µM blends resulted in similar lipid

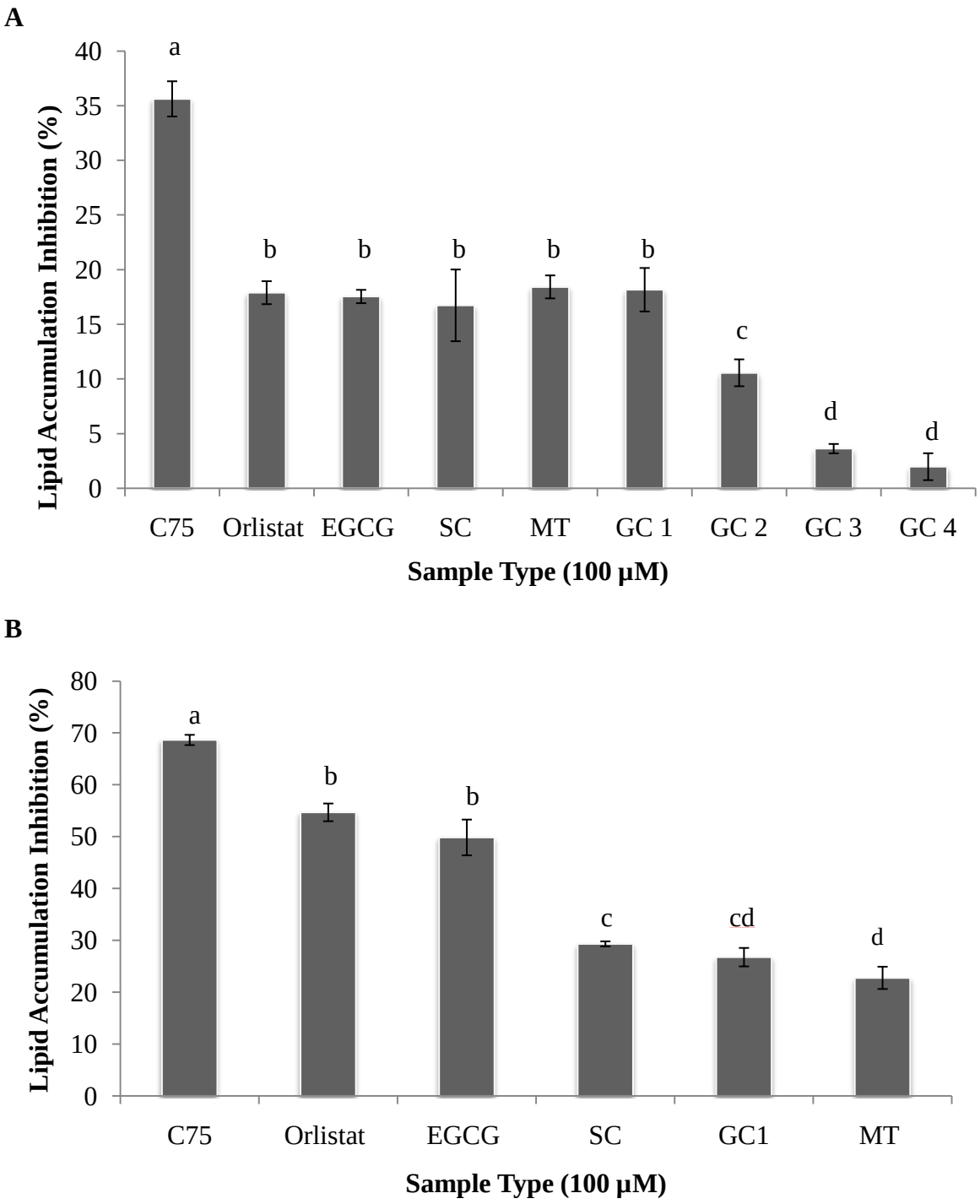
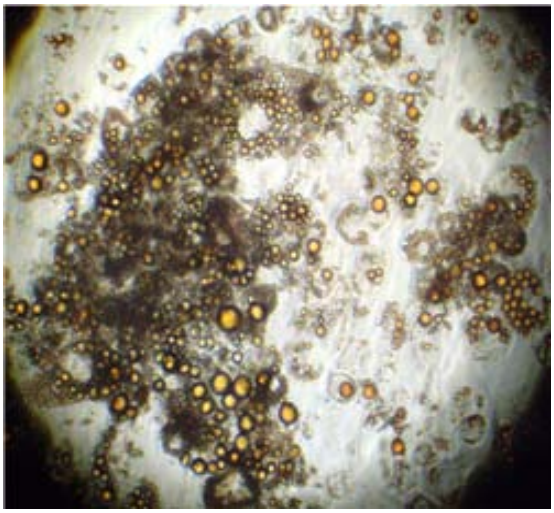
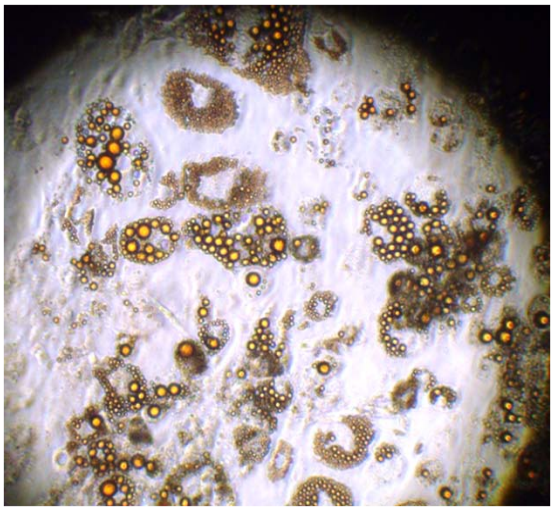


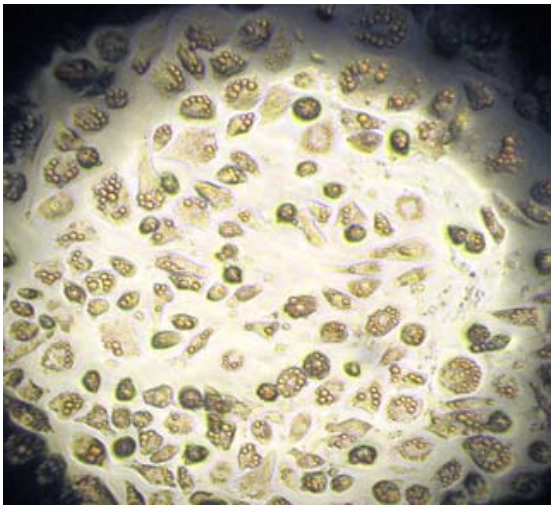
Figure 14. Inhibitory effect of lipid accumulation in 3T3-L1 mature adipocytes. (**A**) After 48 h treatment with MT, GC1, GC2, GC3, and GC4 GC1 in comparison to SC and several positive controls including C75, Orlistat, and EGCG. (**B**) After treatment throughout their differentiation on days 3, 5, 7, and 9 with MT and GC1 in comparison to SC and several positive controls including C75, Orlistat, and EGCG. Bars represent means ± standard deviation based on data from three independent experiments. Different letters indicate significant difference ($p < 0.05$).

A **B**





C **D**



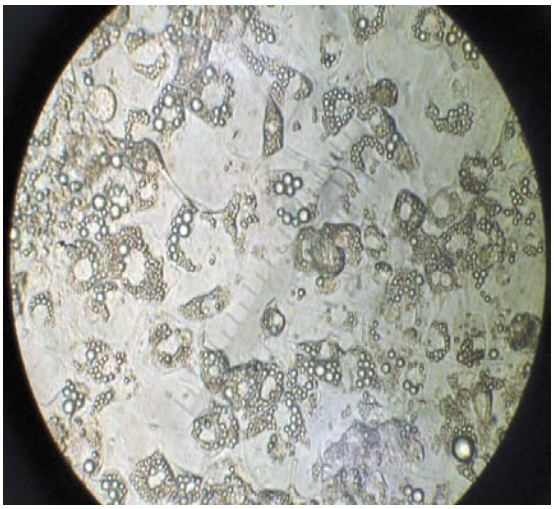
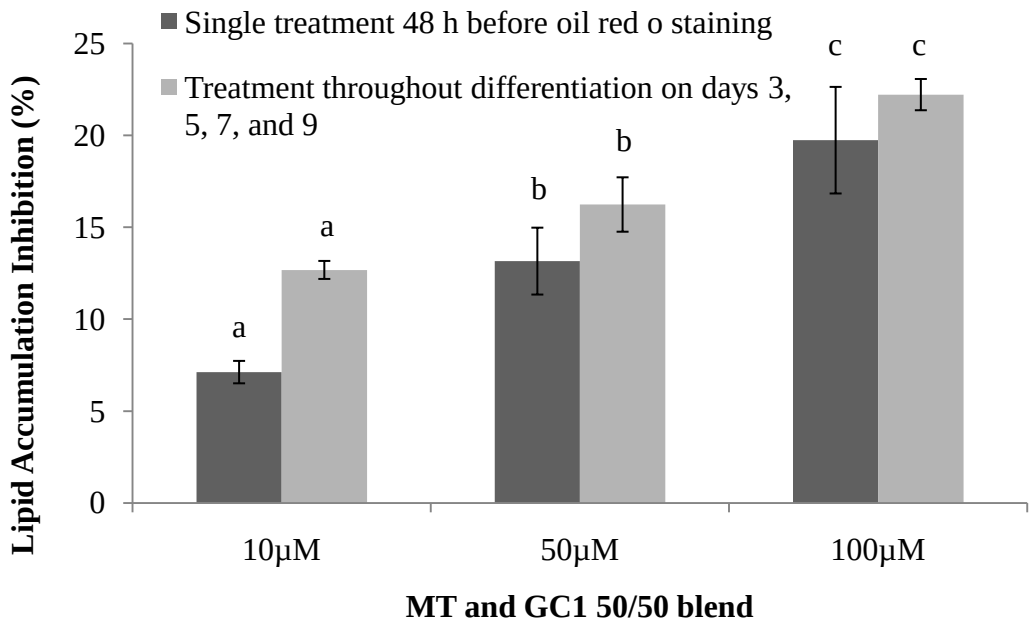


Figure 15. Oil Red O stained material of 3T3-L1 adipocytes treated with (**A**) control (untreated cells) compared to the reduction in lipids in 3T3-L1 adipocytes that were treated at maturity with (**B)** 100 µM MT, (**C**) 100 µM GC1, and (**D**) 100 µM GC1/MT blend.

A



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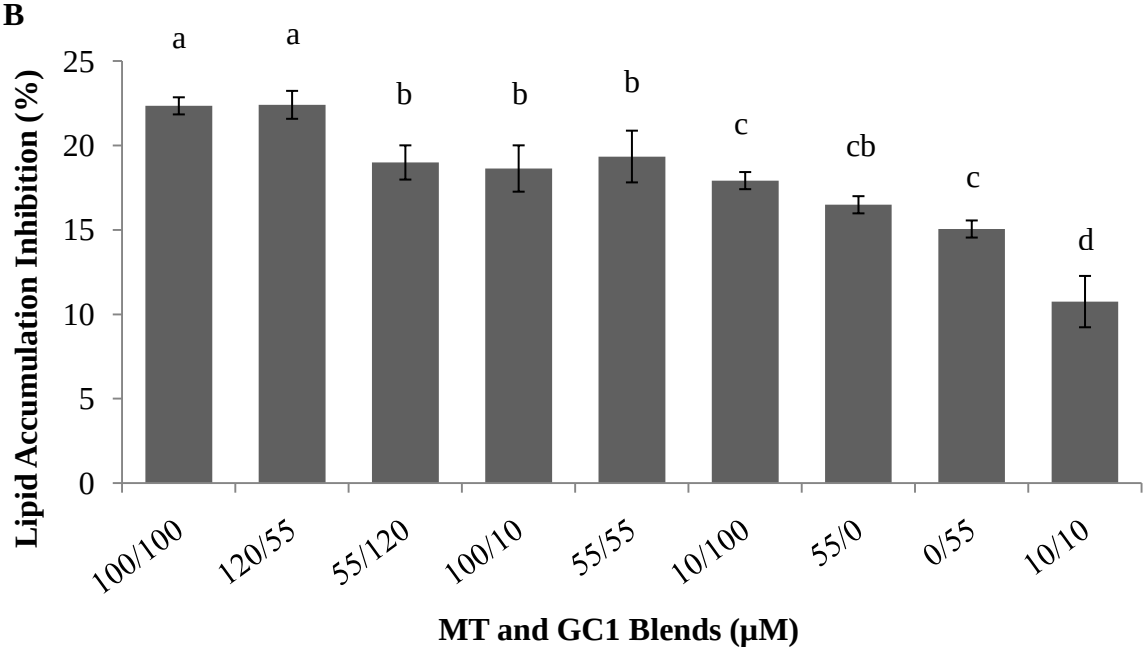


Figure 16. Inhibitory effect of lipid accumulation in 3T3-L1 adipocytes. (**A**) After 48 h treatment compared to treatment throughout differentiation on days 3, 5, 7, and 9 with a 50/50 blend of MT and GC1. (**B**) After 48 h treatment with a variety of blend combinations of MT and GC1. Bars represent means ± standard deviation based on data from three independent experiments Different letters indicate significant difference ($p < 0.05$).

reduction after single treatment as well as when treatedthroughout differentiation. The 100 µM blend resulted in 19.7% ± 2.9 inhibition after single treatment and 22.2% ± 0.8% after treatment throughout differentiation. These results were similar to the results of the individual compounds, concluding that when in combination there was not antagonistic effects. Furthermore, **Figure 16B** depicts the lipid inhibitory effects in mature adipocytes after 48 h treatment with additional blend combinations of MT and GC1. The largest lipid reduction was seen among the blends with the highest concentrations, MT and GC1 blend 100/100 µM and 120/55 µM resulted in 22.4 ± 0.5% and 22.4 ± 0.8% inhibition, respectively. The 55/55 µM blend had a lipid inhibitory effect of 19.4 ± 1.5% which was significantly comparable to the 55/120 µM blend with a 19.0 ± 1.0% inhibition. Additionally, the 55/55 µM blend had the same lipid reduction as the 10/100 µM. These results suggest that at an equimolar concentration it does not matter which byproduct is more or less abundant because comparable lipid reductions will result. Blend combinations of GC3 and MT were also investigated to determine whether or not a synergistic effect would occur as a result of the high polyphenol concentration and antioxidant capacity of GC3. **Figure 17** shows the lipid inhibitory effects seen in mature adipocytes after 48 h treatment of the GC3 and MT blend combinations. The GC3 and MT blend combinations did not result in a synergistic effect. The blend combinations with higher concentrations of MT resulted in higher lipid reductions. The sample which consisted of only 55 µM GC3 resulted in 0.78 ± 0.8% lipid inhibition, whereas the 55 µM MT resulted in 13.2 ± 0.9%. The 55/55 µM blend of GC3 and MT showed a decreased lipid reduction compared to MT alone suggesting that when GC3 and MT are in combination a potential antagonistic effect is present. The results from this study showed

that MT, GC1, and a MT/GC1 blend had a similar inhibitory effect as SC on the intracellular lipid accumulation in 3T3-L1 adipocytes suggesting that these compounds may play a role in lipid synthesis.

3.3 Fatty Acid Synthase (FAS) and Lipoprotein Lipase (LPL) Gene Expression

Figure 18 shows the effect that the caffeinated compounds had on FAS (**A**) and LPL (**B**) gene expression in 3T3-L1 adipocytes relative to the control (untreated cells). FAS and LPL activity were both decreased in 3T3-L1 cells treated with 100 µM SC, MT, and GC1 relative to control a 27.5, 29.4, and 40.9% reduction was seen for FAS

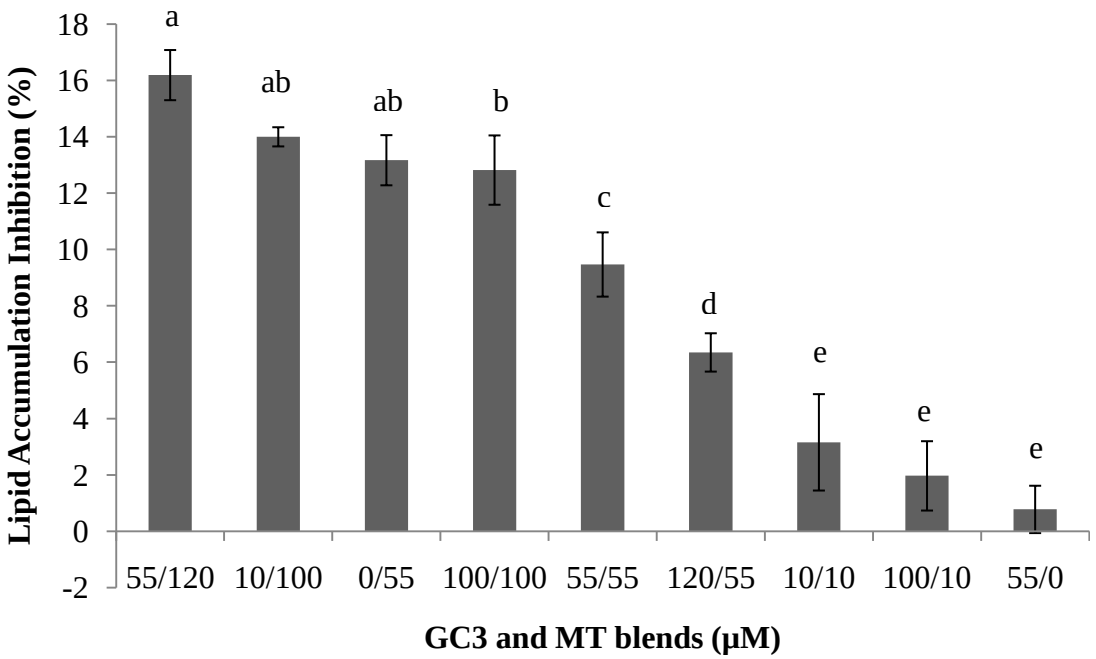


Figure 17. Inhibitory effect of lipid accumulation in 3T3-L1 adipocytes after 48 h treatment with a variety of blend combinations of GC3 and MT. Bars represent means ± standard deviation based on data from three independent experiments. Different letters indicate significant difference ($p < 0.05$).

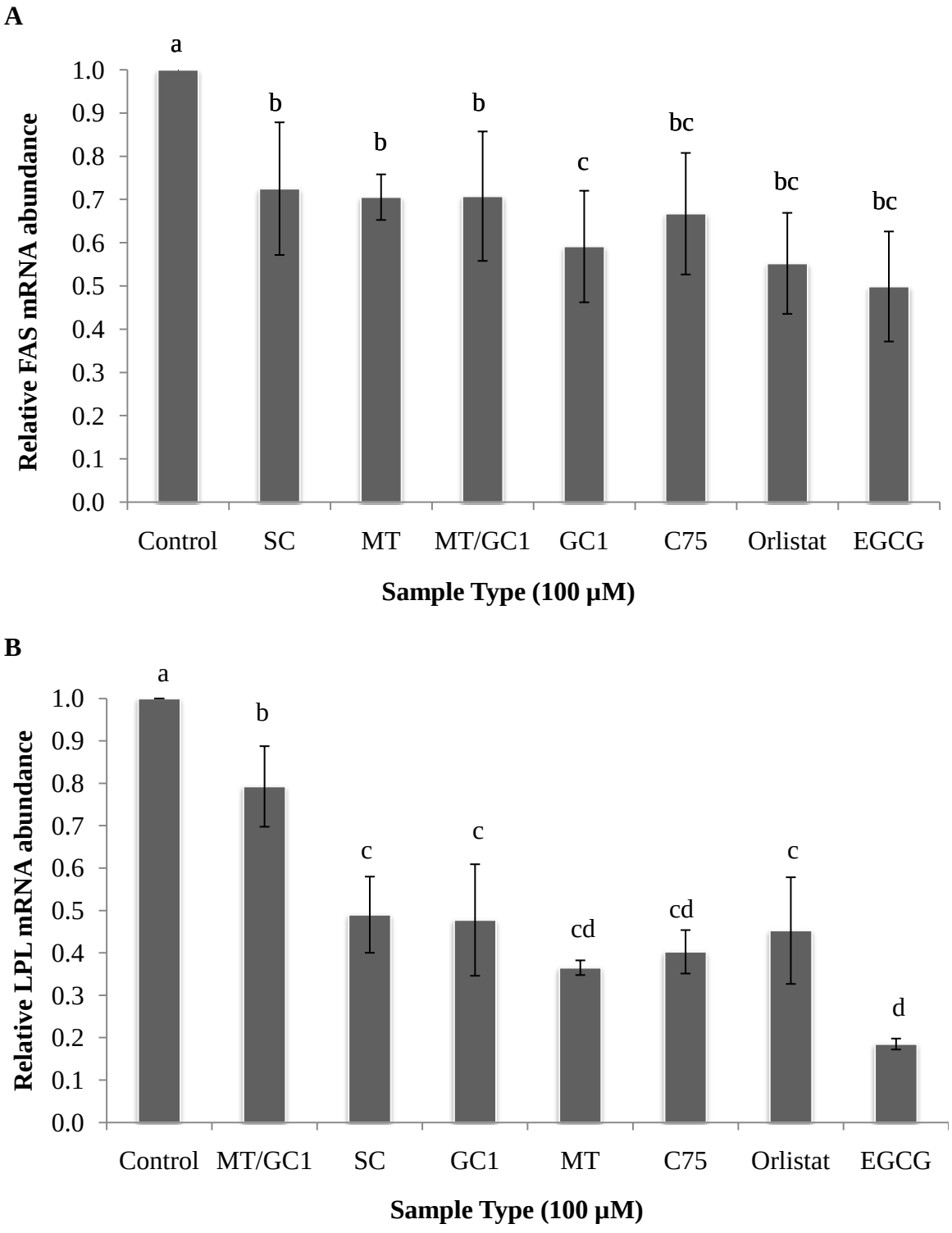


Figure 18. mRNA abundance relative to control in 3T3-L1 mature adipocytes. (**A**) FAS expression inhibition; (**B**) LPL expression inhibition. Bars represent means ± standard deviation based on data from three independent experiments. Different letters indicate significant difference ($p < 0.05$).

expression and a 51, 52.3, and 63.5% reduction was seen for LPL expression, respectively. The results show that all of the tested products altered the gene expression of FAS and LPL, however no correlation was seen with regard to the Oil Red O staining results concluding that other mechanism of action are present. FAS is an enzyme that catalyzes the synthesis of saturated fatty acids and it has been found that the suppression of FAS can lead to dramatic weight loss (Loftus and others, 2003; Schmid and others, 2005). Previous research in this laboratory has shown a down regulation of FAS in rats who consumed a high fat diet with the addition of SC and MT, resulting in suppressed weight gain, concluding that MT is a potential regulator of adipogenesis and lipid metabolism (Zapata, 2007). Another study found FAS activity to be reduced in rats who consumed a coffee bean extract containing 10% caffeine which also resulted in a suppressed body weight gain compared to the rats fed the control diet containing none of the coffee bean extract (Tanka and others, 2009). LPL is an enzyme that produces fatty acids which can be used for direct energy or can be stored as triglycerides in adipocytes (Jing-Jing and others, 2008). The suppression of LPL activity in adipocytes can reduce the uptake of fatty acids resulting in less stored triglycerides.

4. Results of Prototype Development in Comparison to Commercial Energy Beverages

MT and GC1 were found to be as effective as SC in *in vitro* lipid accumulation inhibition and would be a natural, economical, and environmentally sound alternative to SC for usage in beverage applications. The energy drink market is embracing natural ingredients seen with many of the beverages incorporating yerba mate, green tea, ginseng, guarana, and a variety of fruits to only name a few. Incorporating MT and GC1 into a functional energy drink formulation that is geared towards weight management

would be ideal. The beverage prototype concept was an on-the-go tea based energy mix that can be incorporated into bottled water for everyday convenience. This beverage concept would be batch mixed and packaged in individual packets having a 24 month shelf life from date of manufacturing. The beverage mix contains the blend of MT and GC1, formulated to contain 130 mg caffeine/500 ml, which is the typical water bottle size, as indicated in **Table 2**. That caffeine concentration falls well below the recommended daily caffeine intake of ≤ 400 mg (equivalent to 6.5 mg/kg bw/d for a 70 kg person) and is similar to an average 8 oz cup of coffee. In addition to the natural caffeine blend, a variety of South American berries, including acaí, maqui and the mortiño berry were incorporated into the formulation to increase consumer product appeal as well as to enhance the overall health functionality of the beverage (**Table 2**). **Figure 19** shows the AC and the TPC of acaí, maqui, and the commercial mortiño berry, with the maqui berry having the highest AC and TPC. The acaí and maqui berries were both freeze dried, whereas the mortiño berry was commercially processed which may explain the lower AC and TPC of the mortiño berry compared the acaí and maqui.

Table 2. Ingredient composition of the prototype energy beverage

Prototype #	mate tea (mg)	MT (mg)	GC1 (mg)	Acaí (mg)	Mortiño (mg)	Maqui (mg)	AC (trolox µM eq.)	TPC (GA µg eq.)
1	1200	50	50	100	100	10	10888.8	2404.1
2	1200	50	50	100	100	0	10433.7	2344
3	1200	50	50	0	0	0	9574.1	1890.2
4	1200	50	50	100	0	0	10225.7	2112.3
5	1200	50	50	0	100	0	10081.2	2082

*All beverages contained 0.25% cellulose gum

The availability and cost of these berries is a necessary component that needs to be taken into account when formulating. Since a berry blend is being used, doing a cost comparison among the berries and incorporating more of the cheaper ingredient would be beneficial in order to minimize ingredient cost. Yerba mate tea was selected to be used as the beverage base due to its high AC and TPC as depicted in **Figure 20**. In addition to the mate tea, MT, GC1, and berry powders, a cellulose gum was added to the formulation to act as a suspending agent for the dry beverage mixture. This gum is cold water soluble and was used at the recommended 0.25% usage level allowing the beverage mix to have an extremely rapid dissolution rate upon contact with water and only require a minimal amount of agitation. The freeze dried mate tea, MT, and GC1 are water soluble; however the berry powders were not. The gum allowed for consistent particle dispersion of the uniformed small particle sized berry powder to be maintained in a homogenous mixture when put into solution. **Table 2** lays out the ingredient composition of the prototype energy drinks that were developed. All beverage prototypes included the same concentrations of freeze dried mate tea and the natural caffeine sources (MT and GC1), however, the berry combinations varied among the samples. Prototype 1 included all 3 berries, where as prototype 2 included the acaí and mortiño, and prototype 3 included none of the berries. Beverage prototype 4 included only the acaí berry and prototype 5 included just the mortiño berry. The 1.2 g freeze dried mate is equivalent to 5 g of dry mate prepared in 500 ml water, a typical North American usage level. The maqui berry was used at a lower concentration compared to the acaí and mortiño berry because it is a potent natural colorant. The maqui and acaí did not contribute to the beverages overall color therefore prototype 4 and 5 which did not include the maqui

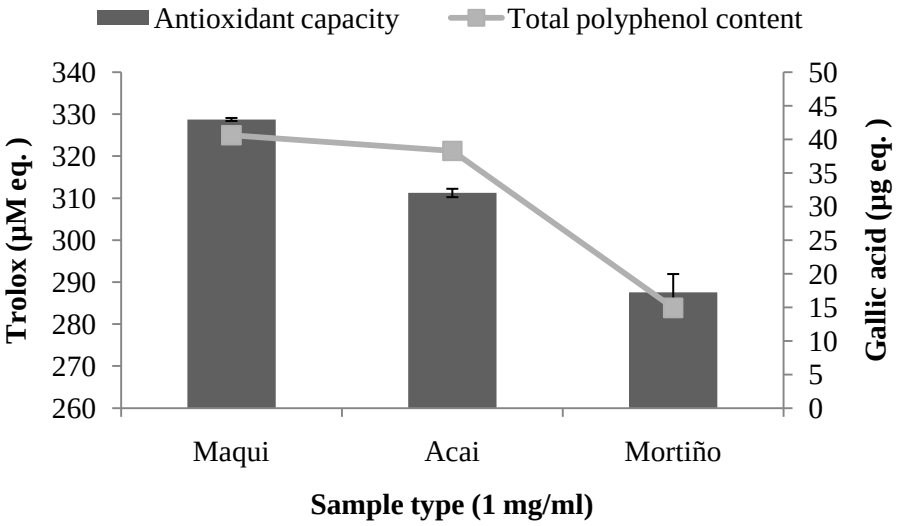


Figure 19. Antioxidant capacity and total polyphenol concentration among the berry samples used in the energy drink formulation.

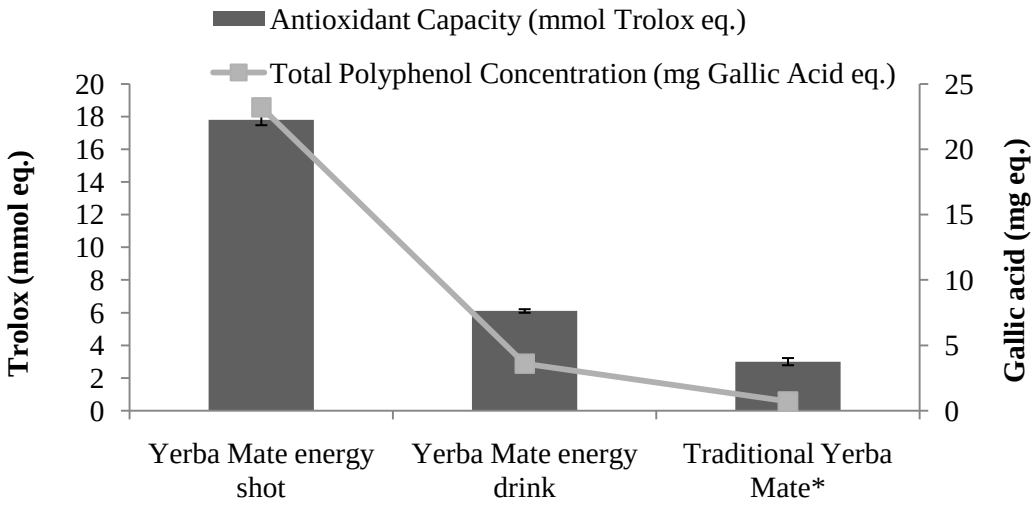
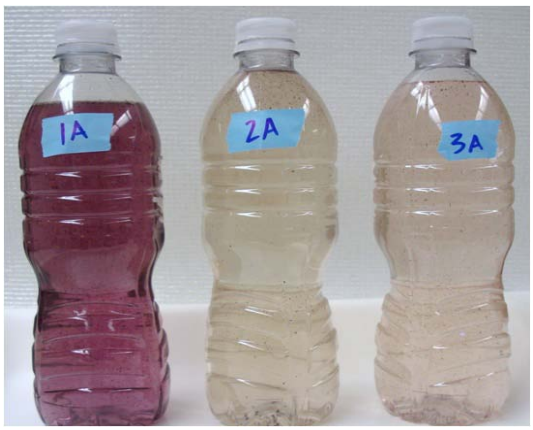


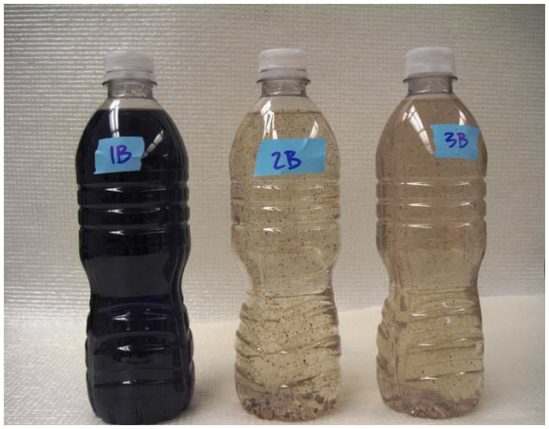
Figure 20. Antioxidant capacity and total polyphenol concentration is 100 fold higher in yerba mate tea based beverages compared to mainstream energy drinks. * The traditional yerba mate tea was prepared by mixing 10 g of tea with 1 L boiling water at 98°C and held/stirred for 10 min then filtered (Chandra and Gonzalez de Mejia, 2004).

would not be visually acceptable products. After these prototype mixtures were added to 500 ml of water the powder rapidly went into solution and a deep blue color resulted due to the anthocyanins present in the berries. Prototype 1, 2, and 3 were also formulated to include 20 mg citric acid which converted the beverage color to a deep red/purple. **Figure 21** shows a visual representation of the 3 beverage prototypes (1, 2, and 3) in which (**A**) represents the beverages with citric acid (**B**) without citric acid, and (**C**) a close up of the most visually appealing and marketable beverage (prototype 1A) in which further analysis was conducted to determine its AC and TPC. Among all of the samples it is apparent that maqui is the only berry that delivers any significant color to the beverage, since maqui was only included in prototype 1 and was the only beverage which displayed a visually pleasant color. After the initial prototype was developed which consisted of the freeze dried mate tea, MT, GC1, acaí, mortiño, maqui, citric acid, and cellulose gum it was analyzed for its AC and TPC and compared to commercialized mainstream energy drinks. Several mainstream energy drinks, 16 non tea-based and 15 tea-based, were analyzed for their antioxidant content using the oxygen radical absorbance capacity (ORAC) assay. Our data resulted in an AC range among the non tea-based beverages of 286.3 ± 63.9 to 3393.2 ± 106.1 µM Trolox eq., with an average of 1275.4 µM Trolox eq. The tea-based energy drinks had much higher antioxidant capacities with a range of 1749.8 ± 212.7 to 45824.2 ± 1430.6 µM Trolox eq., with an average of 10597.9 µM Trolox eq. The AC for the prototype beverage was 10888.8 ± 263.4 µM Trolox eq., which is significantly higher compared to all of the mainstream energy drinks that were analyzed. The TPC was measured in the same energy drinks with the Folin-Ciocalteu method**.** The TPC among the non tea-based beverages ranged

A



B

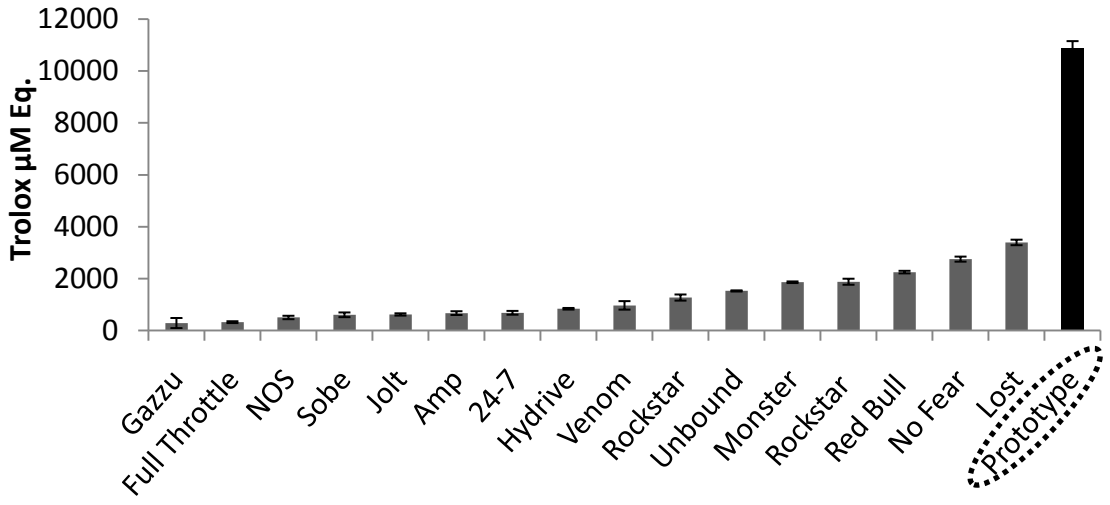


C

Figure 21. Visual representation of the beverage prototypes 1-3. All beverages contained 1.2 g mate tea and 50 mg of both MT and GC1, however, the berry combinations varied. Beverage 1 contained acaí, maqui and mortiño; beverage 2 contained only the acaí and mortiño; beverage 3 contained no berries; (**A**) with citric acid and (**B**) without citric acid and (**C**) a close up of beverage prototype 1A.

from 309.4 ± 1.3 to 1497.5 ± 68.5 µg GA eq. with an average of 673.2 µg GA eq. The tea-based beverages had a range of 496.1± 66.3 to 43010.2 ± 18.9 µM GA eq., with an average of 1318.7 µM GA eq. The TPC for the prototype beverage was 2404.9 ± 38.91 µM GA eq., which was significantly higher compared to the mainstream energy drinks that were analyzed. **Figure 22** shows a comparison of the AC among the (**A**) mainstream energy drinks and (**B**) tea based energy drinks compared to the beverage prototype. **Figure 23** shows a comparison of the TPC among the (**A**) mainstream energy drinks and (**B**) tea based energy drinks compared to the beverage prototype. The AC and TPC values for the prototype beverage can be increased if higher concentrations of the mate, natural caffeine sources, or berries are used; however, from a cost perspective increasing the levels might not be ideal.

A



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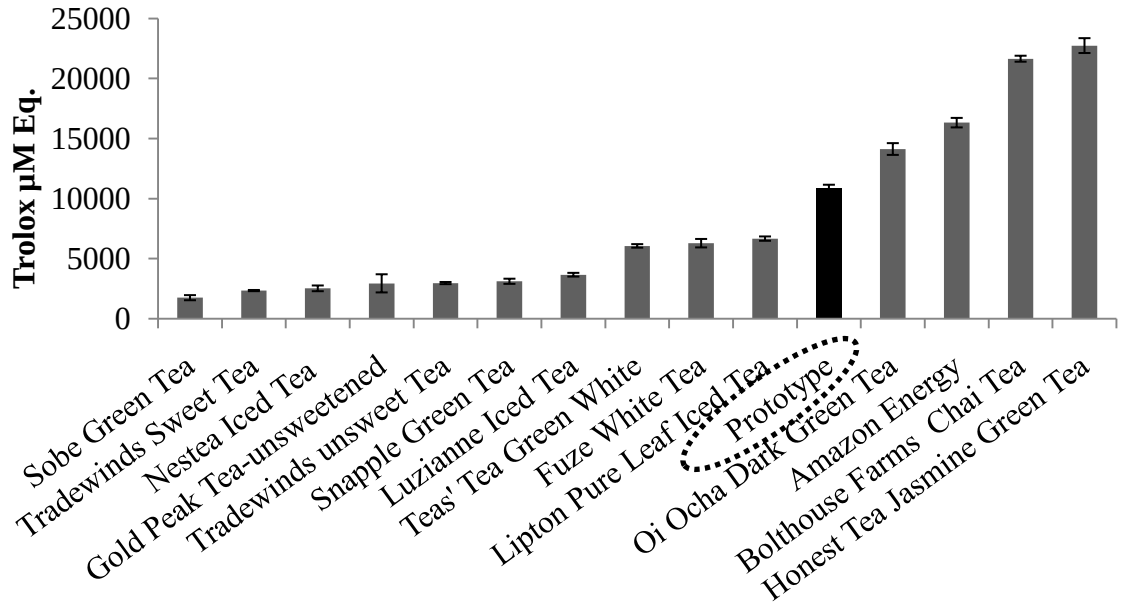
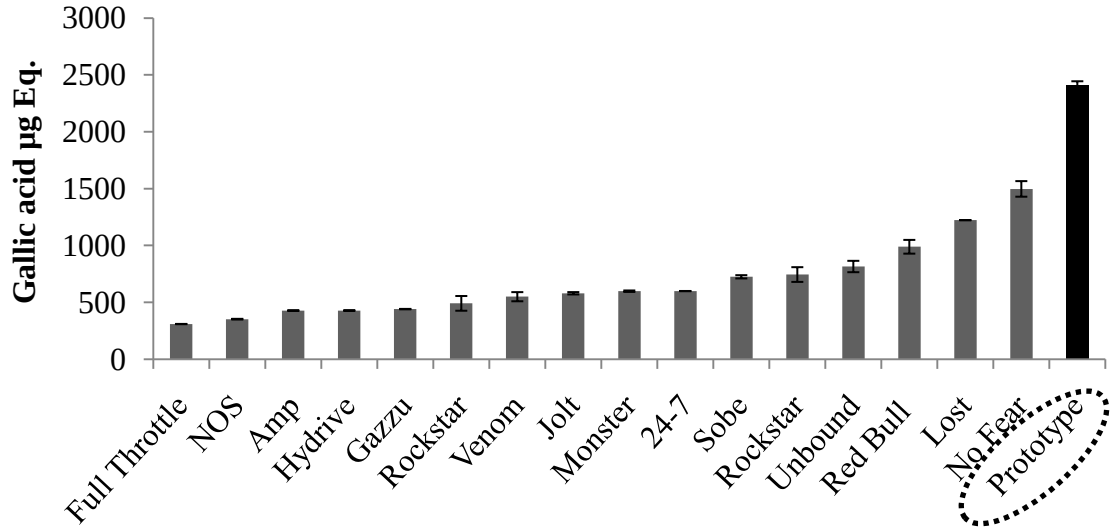


Figure 22. The antioxidant capacity of (**A**) mainstream energy drinks and (**B**) tea based beverages compared to the developed beverage prototype.

A



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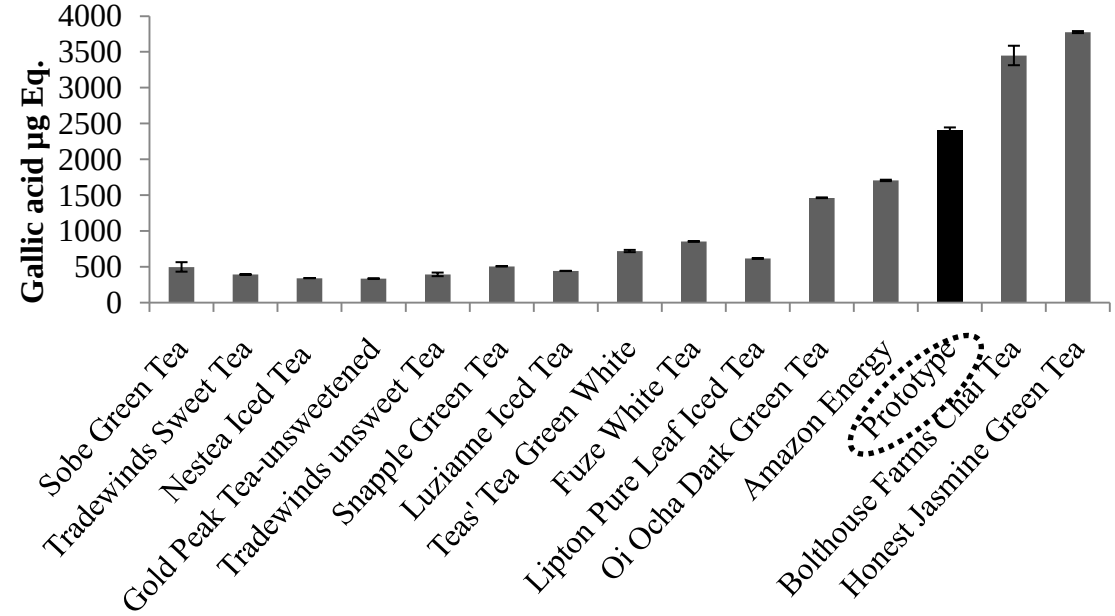

Figure 23. The total polyphenol concentration of (**A**) mainstream energy drinks and (**B**) tea based beverages compared to the developed beverage prototype.

VII. SUMMARY AND INTEGRATION

The prevalence of obesity is increasing worldwide fueling the need for the development of functional beverages geared towards weight management. With the energy drink market growing at an exponential rate, improving the health functionality within that beverage category would be ideal. Caffeine inhibits the lipid accumulation in adipocytes, thus contributing to preventing obesity. Replacing synthetic caffeine (SC) with natural sources like matein (MT) and green coffee (GC) byproducts could aid towards having more health focused products. Additionally, increasing the antioxidant and polyphenol contents of energy drinks would enhance the beverages overall health properties which can be accomplished by incorporating ingredients like Yerba mate and fruits such as the acaí and maqui berry. The increased health functionality of these beverages will benefit the consumer as well as potentially prove to be key drivers for increased sales.

The results from this study suggest that MT and GC1 will promote weight management through the regulation of adipogenesis and lipid accumulation, in a similar manner to SC. The incorporation of MT and GC1 into food or beverage products could potentially decrease adipocyte accumulation and might prove to be a very effective approach for improving long term weight maintenance. However, the clinical significance of these *in vitro* results needs to be validated *in vivo*.

In addition to the lipid reduction, MT and GC1 have a similar AC compared to BHT, a known antioxidant, whereas SC was found to have none. Furthermore, the total polyphenol content (TPC) was determined, and GC1 had a TPC of 9.1 µg GA eq./mg and MT 13.7 µg GA eq./mg, which was over twice as high as SC (5.7 µg GA eq./mg).

These results show great promise for MT and GC1 to be natural alternatives to SC and their incorporation into beverage applications could enhance the products overall health functionality.

A prototype on-the-go tea-based energy drink mix was developed for the incorporation into bottled water which incorporated freeze dried Yerba mate tea, MT and GC1 into the product formulation. The energy drink formulation contained 130 mg caffeine/500 ml, in line with other commercial caffeinated beverages. Additionally, a blend of berries native to South America were incorporated into the beverage mixture which included acaí, maqui and mortiño berries. The berry inclusion improved the AC and TPC of the beverage, proved to be a natural colorant, and enhanced the overall product appeal. The prototype was found to have significantly higher concentrations of both antioxidants and polyphenols compared to mainstream energy drinks and aligned with commercially available tea-based energy drinks.

VIII. CONCLUSIONS

- GC1 and MT were found to have the highest caffeine concentration with values of 94% and 91%, respectively, making them natural alternatives to SC in food and beverage applications.

- GC1 had a TPC of 9.1 µg GA eq./mg and MT was 13.7 µg GA eq./mg; over twice as high as SC (5.7 µg GA eq./mg).

- GC3 had the highest TPC of 153.5 µg GA eq./mg and AC of 1550 ± 19 µM Trolox eq.

- The AC of MT (77 ± 3 µM Trolox eq.) was similar to BHT (71 ± 7 µM Trolox Eq.), a known antioxidant, and GC1 had a reported AC of 34 ± 3 µM Trolox Eq. in an equimolar basis, and SC was found to have no AC.

- The inhibitory effect that MT and the GC byproducts on the lipid accumulation in adipocytes was found to be highly correlated to their caffeine concentration ($y = 5.979x - 4.073$, $R^2 = 0.88$)

- 100 µM of MT and GC1 resulted in a similar lipid inhibitory effect when 3T3-L1 adipocytes were treated at maturity compared to SC, Orlistat, and EGCG; with MT showing the strongest effect of 18.4 ± 1.1%.

- An increased lipid inhibitory effect was present among GC1, MT, and SC after treatment throughout the differentiation process. MT and GC1 resulted in an elevated inhibitory effect, with MT having 22.8 ± 2.2% and GC1 with 26.8 ± 1.8%.

- GC1 and MT in combination suppressed lipid accumulation in 3T3-L1 cells in a dose dependent manner; however it did not show a synergistic but rather an additive effect.

- The results from this study showed that MT, GC1, and a MT/GC1 blend had a similar inhibitory effect as SC on the intracellular lipid accumulation in 3T3-L1 adipocytes suggesting that these compounds may play a role in lipid synthesis.

- FAS and LPL activity were both decreased in 3T3-L1 cells treated with 100 µM SC, MT, and GC1 relative to control; however no correlation was seen with regard to the Oil Red O staining results concluding that other mechanisms of action are present.

- The AC for the prototype beverage which incorporated MT and GC1 was 10888.8 ± 263.4 µM Trolox eq. and the TPC was 2404.9 ± 38.91 µg GA eq., which were both significantly higher than the mainstream energy drinks that were analyzed.

- Commercial mainstream energy drinks averaged 1275.4 µM Trolox eq. (ranged 286.3 ± 63.9 to 3393.2 ± 22.5 µM Trolox eq.) and TPC average was 673.2 µM GA eq. (ranged 309.4 ± 1.3 to 1497.5 ± 68.5 µM eq. GA).

- Commercial tea-based energy drinks averaged 8081.7 µM Trolox eq. (ranged 1749.8 ± 212.6 to 22735.6 ± 618.2 µM Trolox eq.) and TPC average was 1105.0 µM GA eq. (ranged 496.1 ± 66.3 to 3774.9 ± 14.2 µM eq. GA).

IX. FUTURE STUDIES

Future *in vivo* studies are necessary in order to validate the efficacy of MT and GC1 for its potential contribution in weight management through inhibiting adipogenesis and lipid accumulation as were seen in this study in an *in vitro* 3T3-L1 cell model. After validation has been reached, progression towards implementing these ingredients into a functional energy drink beverage application can be carried out. Through this current research a beverage prototype was developed setting the foundation for future studies necessary for product commercialization. The prototype encompasses the MT and GC1, freeze dried Yerba mate tea, a blend of berries, including the acaí, mortiño, and maqui and the appropriate concentrations of citric acid and gums to establish a powder mix that can be added to a water bottle that rapidly goes into solution and stays suspended. Although this drink mix provides 130 mg of natural caffeine and a berry blend that contributes the overall health composition of the beverage, as well as acts as a natural colorant, further research in regards to the sensory characteristics of this beverage needs to be completed. Future sensory evaluations are needed to determine the consumer acceptability of this product as well as to work on flavor enhancement. Additional flavorings or sweeteners may be necessary to mask potential bitter notes contributed by the caffeine, as well as to make the beverage more acceptable to the end consumer.

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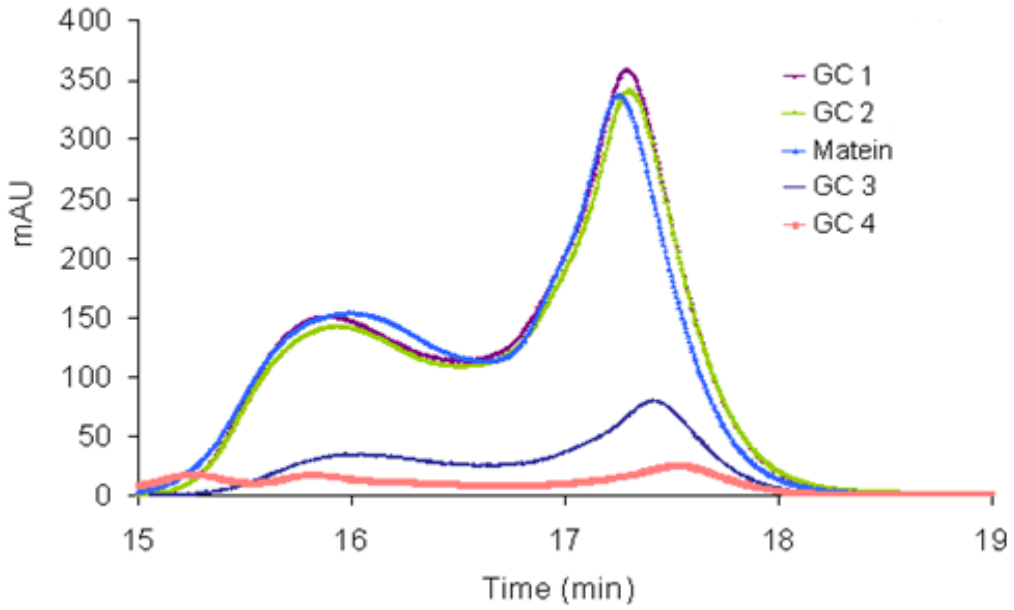
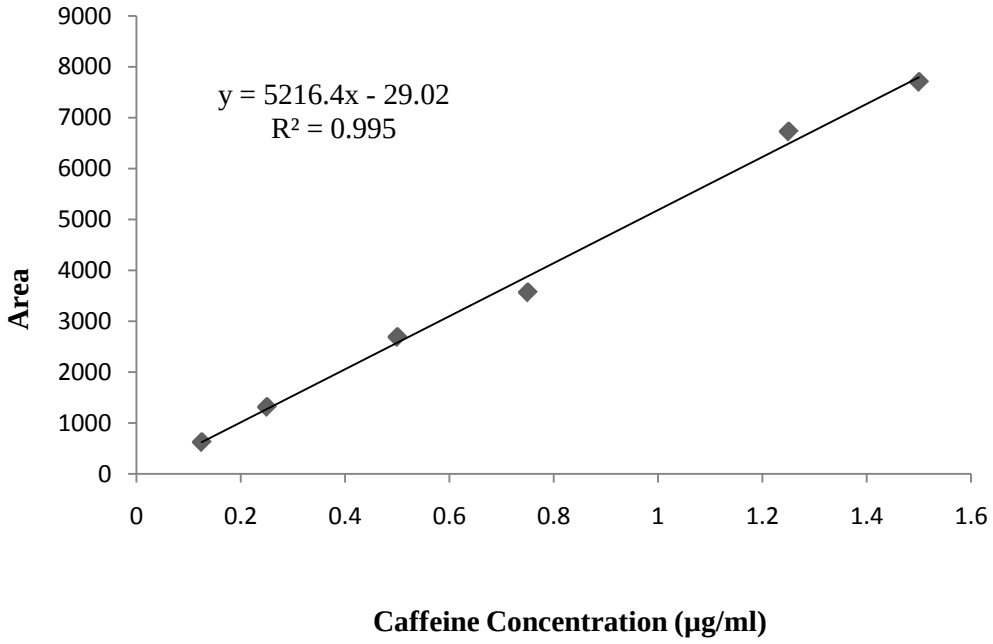
APPENDIX A



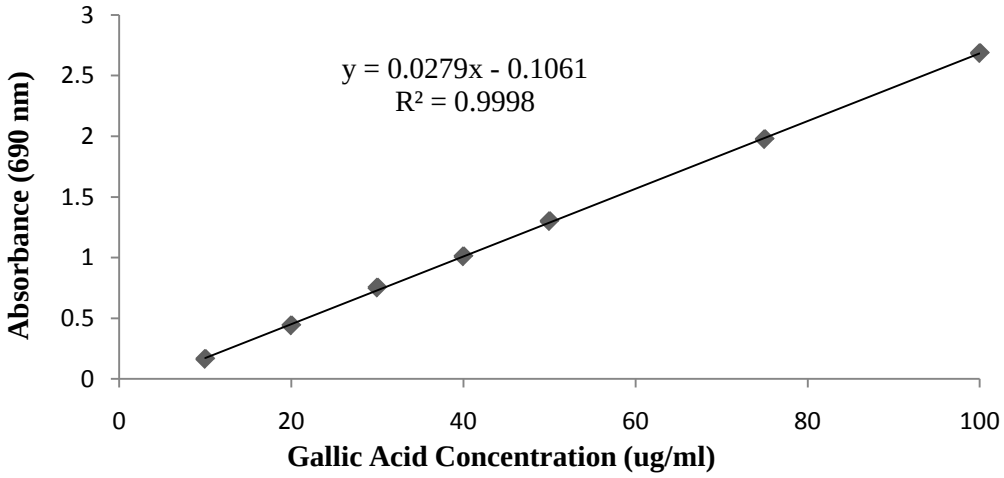
HPLC profile of caffeine in matein, green coffee 1 (GC 1), green coffee 2 (GC 2), green coffee 3 (GC 3) and green coffee 4 (GC4) (synthetic caffeine standard curve: y = 5216.4x – 29.021 R2 = 0.99); abbreviations: **GC1**: green coffee byproduct 1; **GC2**: green coffee byproduct 2; **GC3**: green coffee byproduct 3; **GC4**: green coffee byproduct 4; **MT**: matein.

APPENDIX B



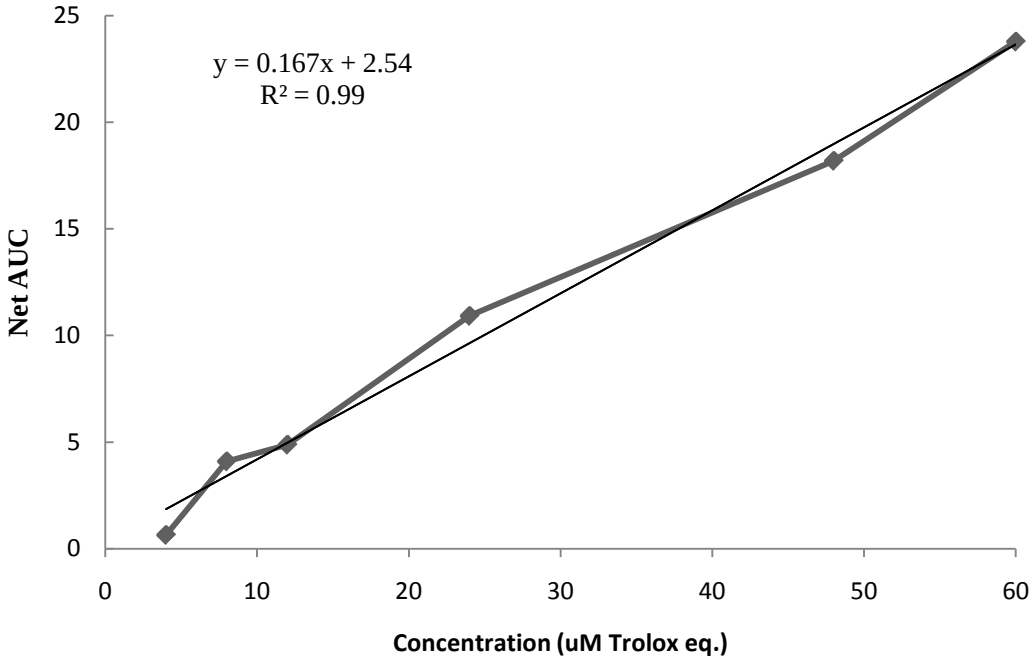
Standard curve of synthetic caffeine at concentrations of 0.125, 0.25, 0.5, 0.75, 1.25 and 1.5 µg after HPLC analysis.

APPENDIX C



Standard curve of gallic acid at concentrations of 0.125, 0.25, 0.5, 0.75, 1.25 and 1.5 µg used for the determination of total polyphenol content.

APPENDIX D



Standard curve of Trolox, a known antioxidant, at concentrations of 4, 8, 12, 24, 48 and 60 µM used for the determination of antioxidant capacity.

APPENDIX E

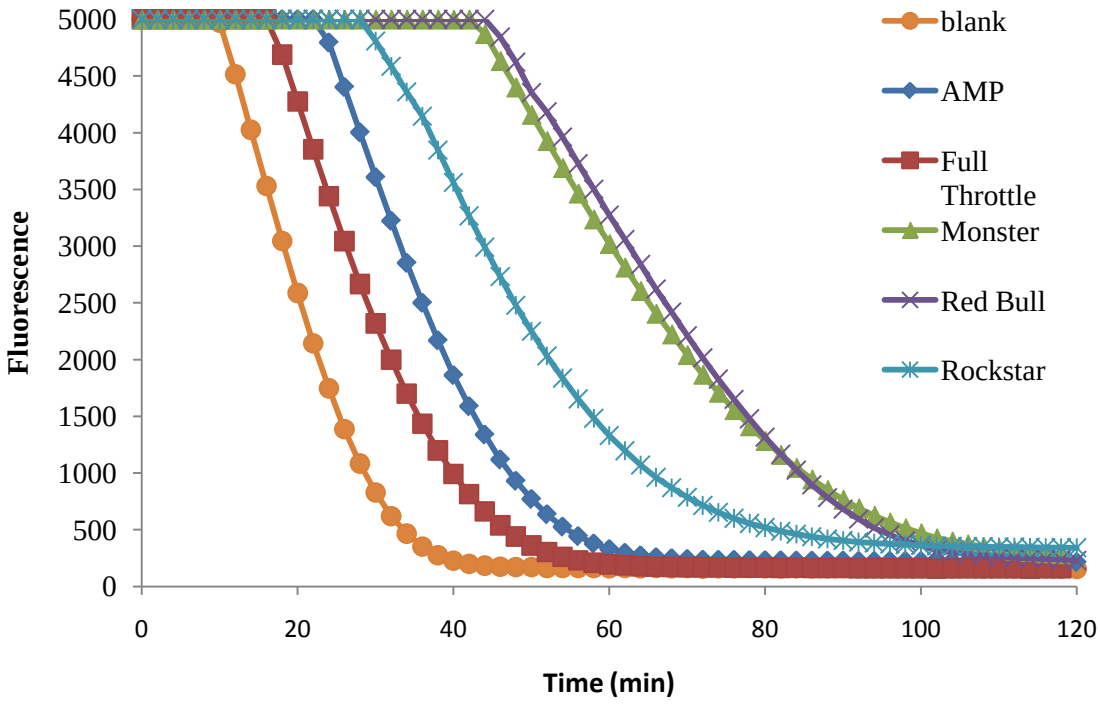
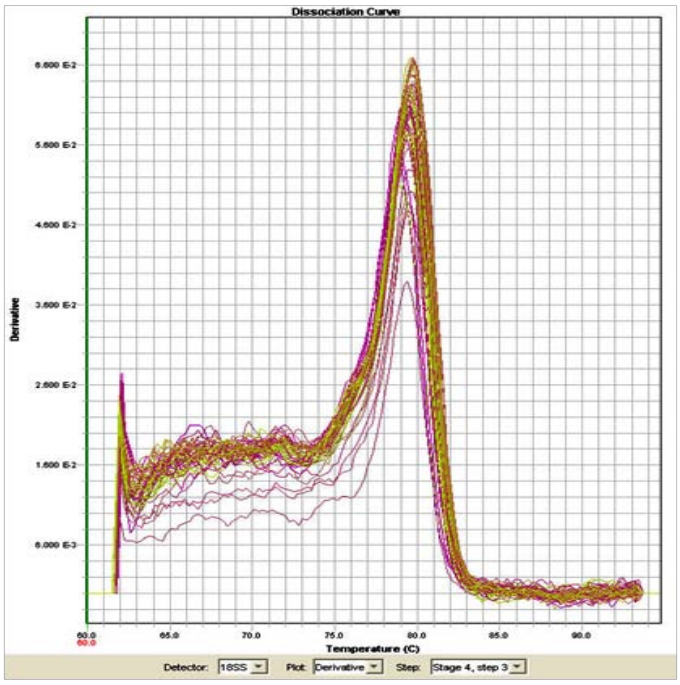


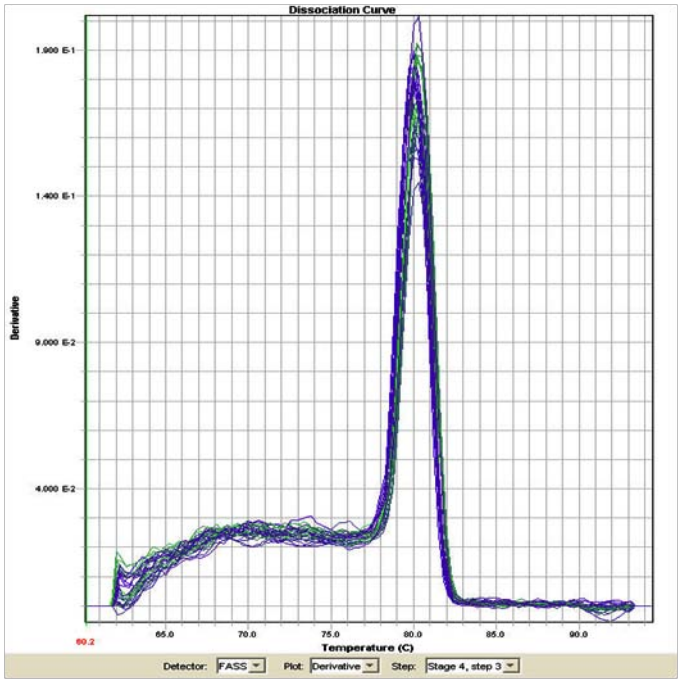
Figure of the fluorescence degradation of the top 5 mainstream energy drinks in the U.S. market compared to the control blank verse time.

APPENDIX F

A



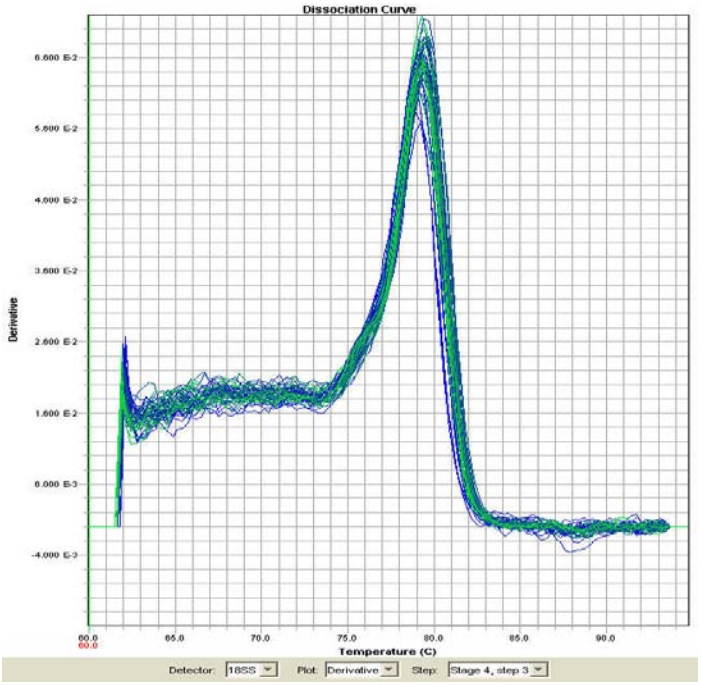
B



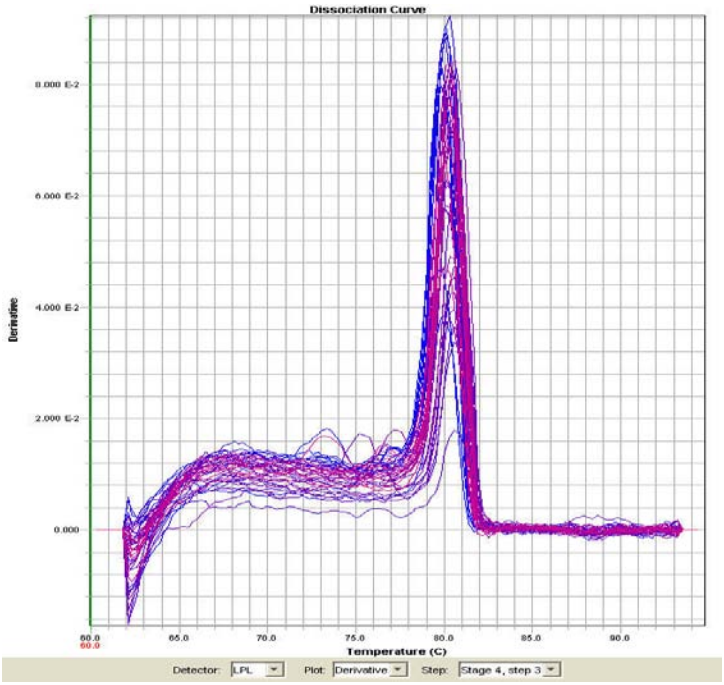
Dissociation curves of 18S (**A**) and Fatty Acid Synthase (FAS) (**B**) mRNA in 3T3-L1 adipocytes

APPENDIX G

A



B



Dissociation curves of 18S (**A**) and Lipoprotein Lipase (LPL) (**B**) mRNA in 3T3-L1 adipocytes

APPENDIX H

Mainstream energy drinks	**Tea-based energy drinks**
24-7	Amazon Energy
Amp	Bolthouse Farms Chai Tea
Full Throttle	Fuze White Tea
Gazzu	Gold Peak Tea-unsweetened
Hydrive	Guayaki Yerba Mate energy drink
Jolt	Honest Jasmine Green Tea
Lost	Lipton Pure Leaf Iced Tea
Monster	Luzianne Iced Tea
No Fear	Nestea Iced Tea
NOS	Oi Ocha Dark Green Tea
Red Bull	Snapple Green Tea
Rockstar	Sobe Green Tea
Rockstar Juiced	Teas' Tea Green White
SoBe	Tradewind’s Sweet Tea
Unbound	Tradewind’s unsweet Tea
Venom	

JFS R: Concise Reviews and Hypotheses in Food Science

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Journal of Food Science

A Publication of the Institute of Food Technologists

Caffeine (1, 3, 7-trimethylxanthine) in Foods: A Comprehensive Review on Consumption, Functionality, Safety, and Regulatory Matters

MELANIE A. HECKMAN, JORGE WEIL, AND ELVIRA GONZALEZ DE MEJIA

ABSTRACT: Caffeine ranks as one of the top most commonly consumed dietary ingredients throughout the world. It is naturally found in coffee beans, cacao beans, kola nuts, guarana berries, and tea leaves including yerba mate. The total daily intake, as well as the major source of caffeine varies globally; however, coffee and tea are the 2 most prominent sources. Soft drinks are also a common source of caffeine as well as energy drinks, a category of functional beverages. Moderate caffeine consumption is considered safe and its use as a food ingredient has been approved, within certain limits, by numerous regulatory agencies around the world. Performance benefits attributed to caffeine include physical endurance, reduction of fatigue, and enhancing mental alertness and concentration. Caffeine has also been recently linked to weight loss and consequent reduction of the overall risks for developing the metabolic syndrome. However, the caloric contribution of caffeine-sweetened beverages needs to be considered in the overall energy balance. Despite all these benefits the potential negative effects of excessive caffeine intake should also be considered, particularly in children and pregnant women.

Keywords: caffeine, fatigue, functional beverages, mental alertness, metabolic syndrome, regulation

Introduction

Caffeine has been used for thousands of years and is one of the most widely consumed active food ingredient throughout the world. It is found in common beverages including coffee, tea and soft drinks, as well as products containing cocoa or chocolate, and a variety of medications and dietary supplements (Barone and Roberts 1996; Andrews and others 2007). Caffeine, the common name for 1,3,7-trimethylxanthine, was derived from the German word *kaffee* and the French word *café*, each meaning coffee. Historians suggest that caffeine was consumed as far back as 2737 BC when Chinese Emperor Shen Nung boiled drinking water and leaves from a nearby bush, creating a pleasant aroma and the first pot of tea (Arab and Blumberg 2008). Coffee originated many years later in the 9th century in Ethiopia when a shepherd began consuming wild coffee berries after observing that his goats had increased energy after eating them (Griffin 2006). It was not until the late 1800's that caffeinated soft drinks began appearing with the introduction of Dr. Pepper, followed by Coca-Cola and then Pepsi-Cola (ABA 2008). The caffeinated soft drink market grew enormously during the 2nd half of the 20th century with increased popularity occurring among the beverages containing higher amounts of caffeine. The increased popularity inspired the arrival of energy drinks, which have become very prevalent. Today, approximately 80% of the world's population consumes a caffeinated product every day and 90% of adults in North America consume caffeine on a daily basis (Ogawa and Ueki 2007). The attractiveness and recognition of these beverages are due to the effect that caffeine has on the body and mind. It has properties that aid in staying awake and improving mental alertness after fatigue (Smit and Rogers 2002). In addition, other findings show that caffeine can be a potential contributor to reducing risk factors involved in the metabolic syndrome, including type 2 diabetes mellitus (DM) and obesity (Westerterp-Plantenga and others 2006; Hino and others 2007). Due to the popularity and wide consumption of caffeinated beverages, the objective of this review was to compile and comprehensively analyze updated scientific information about dietary caffeine, including its consumption, health related functionality, safety, and regulations.

MS 20091104 Submitted 11/4/2009, Accepted 1/13/2010. *Authors Heckman and de Mejia are with Dept. of Food Science and Human Nutrition, Univ. of Illinois Urbana-Champaign, IL, U.S.A. Author Weil is with School of Medicine, University of Buenos Aires, Argentina. Direct inquiries to author de Mejia (E-mail: edemejia@illinois.edu).*

Sources of Caffeine

Caffeine is a naturally occurring alkaloid that is found in varying quantities in the beans, leaves, and fruits of more than 60 plants. Some common sources of caffeine are the kola nut (*Cola acuminate*), cacao bean (*Theobroma cacao*), yerba mate (*Ilex paraguariensis*), and guarana berries (*Paullinia cupana*); however, roasted coffee beans (*Coffea Arabica* and *Coffea robusta*), and tea leaves (*Camelia siniensis*) are the world's primary sources of dietary caffeine (Barone and Roberts 1996). In the United States, most of all dietary caffeine consumed is imported in the form of coffee and tea; cocoa, kola nuts and synthetic caffeine account for a small portion (Bonita and others 2007; Frary and others 2005). There is no chemical difference between synthetic caffeine and naturally sourced caffeine. Caffeine is consumed most frequently in beverages such as coffee (71%), soft drinks (16%), and tea (12%) (Channel Check 2008). The market for caffeinated beverages has increased in the past decade with the introduction of functional beverages, including the energy drinks category, as well as other beverages such as caffeinated sport drinks, juices, and waters (Channel Check 2008). In addition to these beverages, caffeine is also found in cocoa, chocolate, and in a variety of medications such as in some pain reliever formulations and in dietary supplements.

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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Energy Drinks: An Assessment of Their Market Size, Consumer Demographics, Ingredient Profile, Functionality, and Regulations in the United States

M.A. Heckman, K. Sherry, and E. Gonzalez de Mejia

ABSTRACT: The consumption of energy drinks is rapidly increasing, as demonstrated by their large market growth. The targeted demographic group is teenagers, young adults, 18 to 34 y old; although, expansion into nontraditional markets is also occurring. It is claimed that energy drinks can offer an increased energy boost related to their ingredient profile of caffeine, taurine, herbal extracts, and vitamins. Research suggests that energy drink formulations, in addition to increasing energy utilization, may also improve mood, enhance physical endurance, reduce mental fatigue, and increase reaction time. However, in most cases, the corresponding mechanisms of action are not clear. In addition, concerns have been raised over their safety and with a currently weak regulatory environment; efforts need to be made to ensure consumer safety. The objective of this article is to review the current U.S. energy drink market with emphasis on its market size, target demographic, active ingredients, potential benefits, safety, and regulations.

Introduction

Energy drinks refer to beverages that contain, besides calories, caffeine in combination with other presumed energy-enhancing ingredients such as taurine, herbal extracts, and B vitamins. They first appeared in Europe and Asia in the 1960s in response to consumer demand for a dietary supplement that would result in increased energy (Reissig and others 2009). In 1962, a Japanese company, Taisho Pharmaceuticals, launched Lipovitan D, one of the very 1st energy drinks, which is still dominating the Japanese market. Lipovitan D contains B vitamins, taurine, and ginseng, which are all frequent constituents of mainstream energy drinks with the intended purpose of providing the consumer with sustained energy, and to reduce mental and physical fatigue (Taisho Pharmaceutical Co. Ltd. 2009). Energy drinks did not make their way into the U.S. market until 1997 when Red Bull was first introduced, which originated and was initially launched 10 y earlier in Austria (Reissig and others 2009). Since the 1960s, the energy drink market has grown into a multibillion dollar business which has been reported as being the fastest growing segment in the beverage industry since bottled water (Agriculture and Agri-Food Canada 2008). Energy drinks have established a viable position in the beverage market as evidenced by their commonplace

MS 20091110 Submitted 11/5/2009, Accepted 1/15/2010. Authors are with Dept. of Food Science and Human Nutrition, Univ. of Illinois Urbana-Champaign, IL 61801, U.S.A. Direct inquiries to author de Mejia (E-mail: edemejia@illinois.edu).

CURRICULUM VITAE

MELANIE HECKMAN

EDUCATION

- University of Illinois at Urbana-Champaign
  Masters of Science in Food Science and Human Nutrition, May 2010
- University of Illinois at Urbana-Champaign
  Bachelor of Science in Food Science and Human Nutrition with Chemistry minor, December 2007

WORK EXPERIENCE

Research Assistant in Dr. Elvira de Mejia's Lab, University of Illinois, Department of Food Science and Human Nutrition (August 2008-Present)

- Conducted research on the biological activity of green coffee byproducts and the caffeine extracted from yerba mate tea utilizing numerous laboratory techniques
- Developed a natural caffeine blend to replace synthetic caffeine in energy drinks with equivalent effectiveness to inhibit lipid accumulation
- Trained undergraduate and graduate students in various assays and techniques

Teaching Assistant University of Illinois, Department of Food Science and Human Nutrition (January 2009-Present)

- Assisted in the organization, grading and teaching of an 800 student Contemporary Nutrition course
- Facilitated exam reviews, coordinated projects for James Scholar students, managed student questions and office hours
- Coordinated the Student Advisory Committee which facilitated communication between the students and instructor/TAs

Kellogg's Product Development Intern, Battle Creek, MI (January 2008-August 2008)

- Developed flavor formulations and innovative filling concepts for a new food product to enable business growth
- Modified dough formulations to maximize process throughput while maintaining desired sensory characteristics
- Executed more than 20 pilot plant trials and oversaw and helped manage 15 plant workers and production line at 6 full-scale plant trials

Archer Daniels Midland Research and Development Intern, Decatur, IL (Summer 2007)

- Replaced dairy proteins with different lines of ADM soy proteins to reduce costs in several bakery applications while maintaining the same eating quality and appearance
- Investigated new markets and usages for a range of ingredients to increase sales growth

Undergraduate Research Assistant University of Illinois, Department of Food Science and Human Nutrition (October 2005-May 2007)

- Assisted graduate students with their research by running a variety of analytical assays
- Organized and supervised sensory evaluations and consumer panels

SELECTED HONORS AND ACTIVITIES

- Nominee for the Master's research award in the College of Agricultural, Consumer and Environmental Sciences, 2010
- Food Science and Human Nutrition Outstanding Graduate Student Award, 2009
- Kellogg's Scholarship, 2009
- Kathryn Van Aken Burns Merit Award, 2009
- Food Science and Human Nutrition Graduate Programs Committee, Courses and Curriculum (August 2008-Present)
- Lab Seminar Coordinator (August 2008-Present)
- Lab purchasing Coordinator (August 2009-present)
- Facilitated a seminar for Chicago Public High School students on the basics of food science (Fall 2008)
- Institute of Food Technologists (2006-Present)

PUBLICATIONS

Publications:

Heckman MA, Sherry K, de Mejia EG. 2010. Energy Drinks: An assessment of their market size, consumer demographics, ingredient profile, functionality and regulations. Accepted to Comprehensive Reviews in Food Science and Food Safety.

Heckman MA, Weil J, de Mejia EG. 2010. Caffeine (1, 3, 7-trimethylxanthine) in Foods: A comprehensive review on consumption, functionality, safety and regulatory matters. Accepted to Journal of Food Science.

Heckman MA, de Mejia EG. 2010. Comparative *in vitro* lipid reduction and antioxidant capacity of a blend of caffeine from Yerba Mate tea and green coffee byproducts to synthetic caffeine. Submitted to the Journal of Food Science.

Abstracts:

Heckman M, Schreckinger ME, de Mejia EG. Antioxidant capacity, total polyphenol concentration and *in vitro* inhibition of lipid accumulation by selected energy drinks in the U.S. market. Institute for Food Technologists Annual Food Expo Poster Competition. June 2009.

Heckman M and de Mejia EG. Yerba Mate Caffeine (matein) and Green Coffee Byproducts Inhibit Lipid Accumulation in 3T3-L1 Adipocytes *in vitro.* Institute for Food Technologists Annual Food Expo Poster Competition. July 2010.

Research Proposals:

Frankowski K, Heckman MA, de Mejia EG. Effects of natural caffeine sources on lipid accumulation in 3T3-L1 adipocytes. ACES Undergraduate Research Scholarship Project Proposal Spring 2009.

Sherry K, Heckman MA, de Mejia EG. Investigation of Commercialized Energy Drinks and Their Antioxidant Capacity. Cargill Scholarship Fall 2008.

TECHNICAL SKILLS

- Antioxidant capacity
- Cell culture
- Extrusion Technology
- Freeze dryer
- HPLC, LC-MS, GC-O
- Statisitical analysis
- Microsoft Office (Excel, PowerPoint, Publisher, Word)
- NanoDrop Spectrophotometer
- Real-time PCR
- RNA extraction
- Statistical analysis
- HPLC, LC-MS, GC-O
- SYBER Green Assay
  Total polyphenol content

RELATED COURSE WORK

- Advanced Food Microbiology
- Biochemistry
- Flavor Chemistry & Analysis
- Food and Industrial Microbiology
- Food Chemistry I and II
- Food Process Engineering
- Food Processing I and II
- Food Production and Service
- Food Service Sanitation
- Principles of Nutrition
- Product Development
- Raw materials for Processing
- Sanitation in Food Processing
- Sensory Evaluation of Foods
- Statistics
- Toxicology

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